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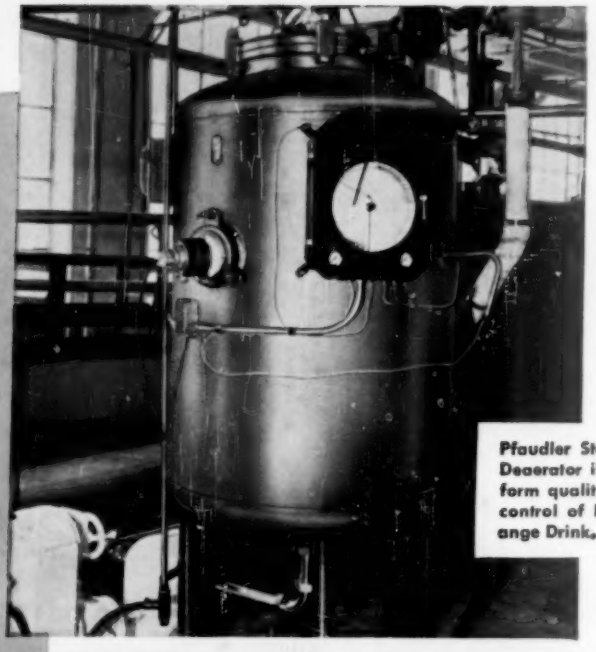
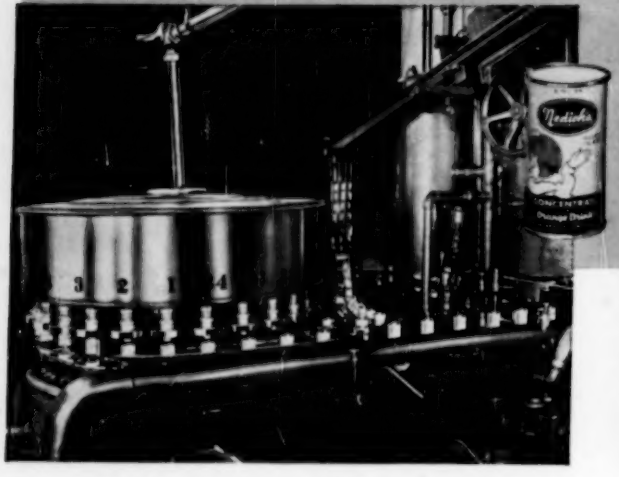
How Pfaudler Equipment Aids Nedicks

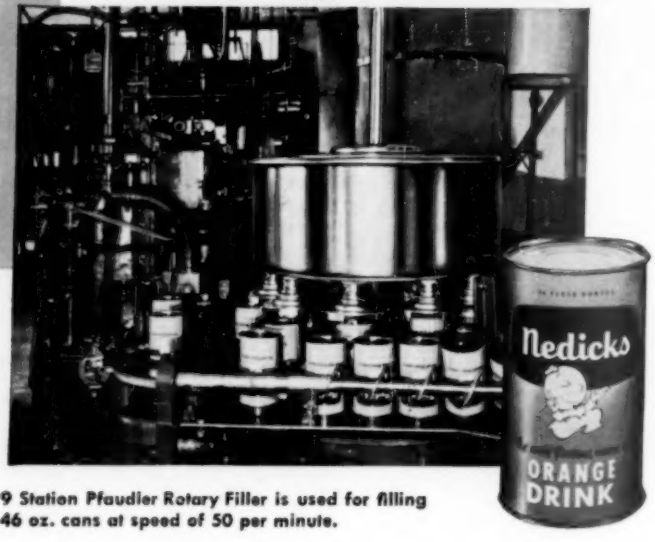
to process quality orange drink



Pfaudler Stainless Steel Deaerator is key to uniform quality and flavor control of Nedick's Orange Drink.



24 Station Pfaudler Rotary Filler where 6 oz. cans are filled at a rate up to 400 per minute.



9 Station Pfaudler Rotary Filler is used for filling 46 oz. cans at speed of 50 per minute.

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EDITORIAL

Some Growth Traits of the Food Manufacturing Industry[a]

ALLEN B. PAUL
University of Illinois, Urbana, Illinois

(Received for publication, May 29, 1950)

During the forty-year period, 1909-48, the U. S. food manufacturing industry expanded at as rapid a rate as general manufacturing, but with more stability over short periods. Conversely, the expansion of food manufactures was more rapid but less stable than expansion of farm food production. The explanation and significance of these comparative growth patterns are examined.

Associated with the expansion of industry are patterns of change in the numbers and size of plants. Recent post-war changes are examined against the background of an idealized long term pattern, and the meaning of the consistency between short term and long term patterns is discussed.

In general, the average size of food plant increased favoring the application of improved technology to manufacturing operations.

This paper attempts to interpret general economic characteristics attending the growth and development of the U. S. food manufacturing industry. It relates to the nature, origin, and significance of (1) changes in the output of food manufacturing services, and (2) changes in the size and number of food manufacturing plants.

CHANGES IN THE OUTPUT OF FOOD MANUFACTURING SERVICES

In the present context, food manufacturing services consist of food processing operations, plus other services incident thereto, performed within food plants. Included are handling, storing, conditioning, processing, packaging, packing, and associated services (such as the services of management, clerks, technicians, maintenance crews, etc.). These are food manufacturing services in the sense that they are united around the processing operations.

Although measurement of changes in the output of food manufacturing services is difficult, such measurements can be made with a reasonable degree of validity for the present purpose. A graphic view of the changes over the forty year period 1909-1948 is shown in Figure 1. The output of food manufacturing services in 1939 was roughly 150 percent greater than in 1909; in 1948, about 270 percent greater than in 1909.

These services are performed on agricultural products. By way of contrast, food production at the farm level increased only 40 percent between 1909 and 1939; and 75 percent between 1909 and 1948. This great disparity in growth rates of the food manufacturing and farm sectors is striking because the two sectors are sequentially related in the over-all productive process.

Factors that limit the expansion of farm food production do not limit the expansion of food manufacturing services. People respond to the preservation,

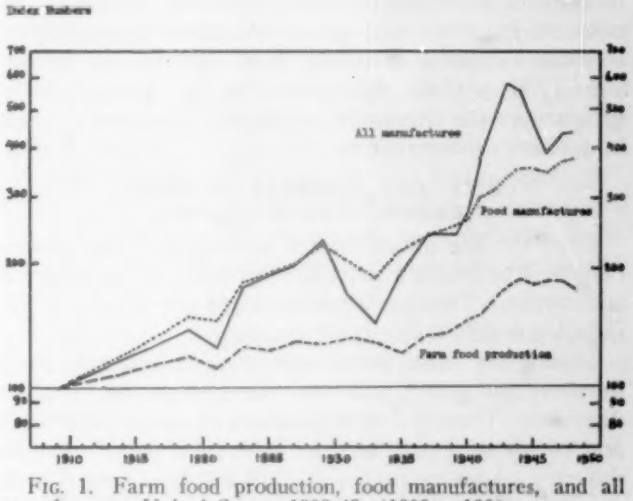

FIG. 1. Farm food production, food manufactures, and all manufactures, United States, 1909-48. (1909 = 100).

refinement, elaboration of foods and related appeals (as convenience and esthetic forms) in a manner that is not circumscribed by physiological limitations.

It is remarkable that the output of food manufacturing services had, in a general way, kept pace with the expansion of all U. S. manufacturing operations. (Figure 1). The latter are not limited by physiological intake tolerances. The long-term trends of food manufacturing and all manufacturing are quite similar over the 1909-1948 period, possibly a unique experience in the sense that it may not be repeated in the next forty years.

However, this similarity suggests that the origins of the growth of food manufacturing services most likely will be found in the socio-economic setting of rising real incomes and technical and industrial expansion. In principal there appear to be no limits to the expansion of food manufacturing services other than those posed by real incomes, consumer preferences, technology, and pricing policies.

There is one modification of some importance. The growth trend in food manufacturing was caused partially by the transference of processing operations from farm and distributive agencies to factories. Measurements indicate that the transference has been one of the minor causes of growth of the commercial food manufacturing sector,—of the order of 20 percent. The current reservoir of transferrable operations is much smaller than it was in 1909; therefore its contribution to the future growth of the commercial sector probably will be of small consequence.

A second feature of the growth pattern relates to stability. The growth of the food manufacturing industries has been more regular than of total manufacturing; but not as stable as that of farm food production.

[a] Presented at the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 24, 1950.

Manufacturing activity is sensitive to prosperity and depression, particularly the production of non-food commodities. The latter embrace capital goods industries, and industries that produce articles that people will readily forego in a depression. Manufacturing services in foods are not as easily given up.

The above analyses have several implications. Important is the share of the consumers' food dollar going to farmers, distributors, and processors. There is no constant share for each group. Returns for processing services increased gradually from 12c to 21c in the 1914-1939 period, due primarily to the relatively greater increase in services rendered by processors than by farmers or distributors.

SIZE AND NUMBERS OF FOOD MANUFACTURING PLANTS

Over the life experience of an industry plant numbers tend to follow a pattern of growth and subsequent contraction. This cycle occurs while the output of the industry is still in its expansion stage.

During the initial growth phase of an industry, plant numbers multiply rapidly and the average size of plant increases. Then the multiplication of plants tapers off and comes to a halt, at the "saturation point" for the industry. A net reduction in plant numbers next occurs, resulting from the withdrawal of the less fortunately situated plants (in all relevant respects) in larger numbers than new plants are created. The average size of plant increases markedly, and from this point on the expansion of the industry takes this form rather than through increases in plant numbers. (Adjustments in plants, — geographical and operational, — go on independently of net changes in plant numbers.)

The above sketch is an idealization of the long-term development. Changes during short-term periods may or may not be consistent with the long-term pattern. Depression periods and war periods are times of marked changes. It is clear the one impact of a major war and its aftermath on food manufacturing industries is to expand their outputs sharply. It is not clear, however, what effect this would have on plant numbers. Presumably this would depend on the usable reserve capacity in existing plants, the relative impacts of government allocative powers on the increase in plant size versus expansion of plant numbers, and other factors. The question then is one of determining what did happen and why.

A first approximation of recent wartime changes can be gleaned from the recent 1947 *Census of Manufactures*. It will be sufficient for present purposes to use approximate measures of changes in industry output (i.e. changes in employment) and changes in plant size (i.e. changes in workers per plant). The changes were so marked that a clear indication of their nature shows up, despite limitations of such measurements. From 1939 to 1947 employment in the food manufacturing industries expanded by 46 percent, in all manufacturing, by 53 percent. Both are substantial increases. During this period the number of food plants declined by 10 percent, whereas the number of all manufacturing plants increased 39 percent. Among the several branches of the manufacturing industry listed by the census, the food sector is the only one that experienced a decline in plant numbers. However, figures for the over-all food industry hide the greatly divergent experiences of individual food processing industries.

The experiences of 38 branches of the food industries for which comparisons can be made for the period from 1939 to 1948 are shown in Table 1. Expansion in output was a feature common to most of them; 32 of the 38 industries increased output by 10 percent or more. Out of the 32 industries, 9 increased primarily by expanding the number of plants with little or no change [c] in the average size of plant; 5 industries expanded output primarily through expanding operations of existing plants with little or no change in plant numbers; while 9 other industries expanded output despite significant decreases in numbers of plants. Eight industries expanded through significant increases in both types.

Here is an interesting area for inquiry. The varied array of behavior patterns should furnish fruitful

TABLE 1

Percentage Changes in Average Number of Workers per Plant, Plant Numbers, and Employment in U. S. Food Processing Industries, 1939 to 1947 [b]

	Workers per Plant	No. of Plants	Employment
	(percentage change from 1939 to 1947)		
Natural cheese	231	−32	124
Ice cream	154	−16	114
Malt liquors	143	−27	77
Bread, cake, rolls	128	−34	50
Flour milling	114	−42	24
Prepared flour	109	56	228
Wines	108	20	149
Poultry dressing	86	−26	38
Sausage, n. e. m.	85	6	96
Butter	69	−38	4
Macaroni, spaghetti, etc.	60	−31	10
Flavorings	59	27	102
Canned and dried milk	59	0	59
Soft drinks	55	25	94
Chewing gum	52	37	108
Chocolate and cocoa	50	−20	19
Biscuits and crackers	40	− 8	29
Malt	39	2	42
Breakfast cereals	39	− 9	27
Beet sugar	28	−13	12
Oleomargarine	22	50	84
Soybean milling	21	183	243
Cane sugar refining	15	− 7	7
Rice milling	14	22	39
Cottonseed milling	13	−30	−20
Canned fruits and vegetables, preserves	9	19	30
Pickled fruits and vegetables, salad dressings	2	45	49
Cured fish	2	11	13
Canned sea foods	1	19	20
Leavening compounds	− 1	2	1
Frozen foods	− 1	377	373
Shortenings	− 1	22	21
Candy	− 3	35	30
Starch products	− 4	57	50
Meat packing	− 6	55	45
Cane sugar mills	− 9	5	− 5
Vinegar and cider	−12	−11	−22
Processed cheese	−71	886	190
Food manufactures, total	61	−10	46
All manufactures, total	10	39	53

[b] Source: Based on data from *U. S. Census of Manufactures*. Figures shown have been rounded to the nearest whole number.

[c] The expression "little or no change" refers to a change of 10 percent or less, an arbitrary limit selected for expository purposes.

subject matter for an understanding of the forces that shape the changing physical structure of food industries. Frozen food manufacture, soybean milling, and prepared flours are relatively new industries and the great increase in plant numbers can be understood in terms outlined above. However, the great increase in processed cheese plants may require another explanation, i.e. patent expiration and other factors. In what terms is the expansion of meat packing plants to be understood?

Similarly the decrease in plant numbers in natural cheese, bread, flour milling, butter, chocolate, cottonseed milling, macaroni, and malt liquors industries may be explained in the long-term industry cycle context, but the manner in which this occurred must be established.

What seems to be fairly clear is a general tendency of food plants to expand their average size of operations. Where the size growth within the range of plant sizes took place should be studied. In any event, this growth tendency favors application of improved technology to the manufacturing operations.

RECENT CHANGES IN SIZE OF FIRMS

Another important trait relates to the increase in size of firms,—the ownership pattern. Firms grow by expanding the operations of their facilities and by acquiring or building additional facilities. The gaps in this area of knowledge are large. A recent study of the growth of manufacturing firms through acquisition of existing plants during 1940-47 shows that acquiring companies in the food sector increased their asset position through this method by some 11 percent. But behind this average figure lies an interesting fact: relatively greater growth (through mergers) occurred among the smaller acquiring companies than among the larger companies (those with assets of 1 to 5 million as opposed to those with assets of over 100 million) *(1)*. Thus, a favorable condition is developing among the smaller firms for the increased application of technological knowledge to food manufacturing operations.

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Problems of Subsistence That May Confront the Quartermaster Corps in the Future[a]

MAJ. GEN. W. H. MIDDLESWART
Military Planning Division, Office of the Quartermaster General, Washington, D. C.

(Received for publication, May 29, 1950)

In the event of another all-out war, certain grave food problems of a general nature must surely arise, no doubt without warning. The rapid developments of long range airborne military attack systems present bitter probabilities if world-wide hostilities begin. The three most apparent problems, or perhaps we should consider them as dilemmas, are, I think, the serious disruption of food transportation facilities, extensive destruction of our food processing plants, and widespread damage to our storage plants, grain elevators and stock yards.

Few Americans nowadays suffer the delusion that our cities are immune to war damage simply because the maps place the United States thousands of miles from a possible enemy country. Modern air warfare techniques all but ignore rivers, mountains, and oceans, and possbily the Arctic wastes as well. Recent airpower developments make an attack across the Arctic Circle a distinct possibility.

If attack comes, whether across the North Pole or otherwise, with guided missiles over thousands of miles, and with atomic bombs, our present concentrated food production centers might well be put out of action in a period of minutes.

For example, the great industrial centers, Chicago, Detroit, Pittsburgh, St. Louis, Kansas City, Omaha, are all within feasible bombing distance from points across the Arctic which, before the age of long-range air operations, were considered too remote to cause us concern. If we add to the destruction of processing plants and transportation facilities, the loss of skilled labor and experienced technologists, we have a disaster of the first magnitude.

The ramparts we watch—where are they? Indeed, *what* are they? Once upon a time bastions were built upward from the ground in the form of walls around cities. They had vertical dimensions, and limits on either side. Today, any defense system must be horizontal, with no more in the way of dimensional limits than has the infinite air which surrounds the world. In the foreseeable future, not one corner of our nation can believe itself immune from destructive assault.

The old ideas about time and distance, and destructiveness, no longer mean very much. The old rules, which told us what to do in case of war, went up in the smoke of the first atomic bomb. Any American who feels our national security and our personal safety are assured by our past accomplishments either cannot read, or is deliberately blind. In this day of supersonic airspeeds, guided missiles, and atomic power, one fact emerges with alarming clarity: Never again will this nation be permitted the luxury of months, not to mention years, to organize for a war after it has started.

As a nation, nothing in our past history has trained us for wide-spread calamity. Patriotic catch-phrases, color food-stamp controls, increased production cam-

[a] Presented at the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 25, 1950.

paigns, prayers for timely rain, these will not get us through. And last, but not the least troublesome is mankind's hoarding instinct which, sadly enough, flourishes in the breasts of the loudest sloganeers.

Food controls are fairly simple to exercise in small areas for small groups. We must plan adequate controls in terms of the nation as a whole, over three million square miles and one hundred fifty million people, accustomed to three square meals, of a very high order, every day.

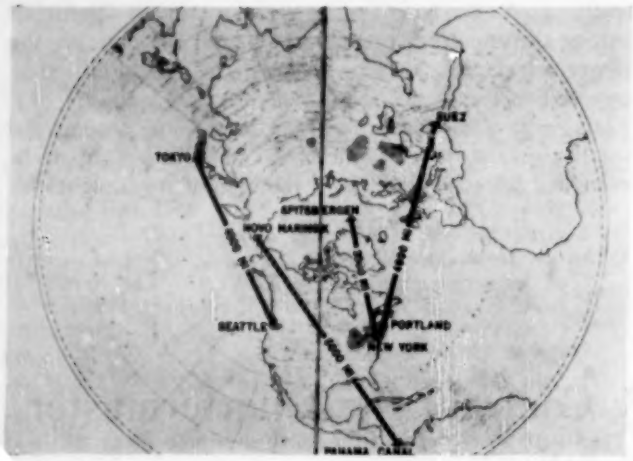

FIG. 1. These routes over the roof of the world are the shortest possible between the given points. Any one of these distances is within the range of super-bombers. (Photo No. RD 10,003. Office of the Quartermaster General, U. S. Army, Military Planning Division.)

We must think in terms of such problems as how to maintain and control the food-supply of both civilian and military personnel under a disrupted food economy; how to process food and transport it under attack; how to get vast stores of military subsistence to military forces in the field; and how to maintain throughout the incredible destruction of modern warfare the well-being and morale of the military forces.

The role that food can play in the maintenance of physical and mental efficiency needs no elaboration here.

Let us look more closely at each of the foregoing broad problems. We shall have, as I have said, the problem of maintaining and controlling the food supply at home.

The magnitude of this job is startling. Thirty-seven percent of all our country's productive resources are destined for our dinner tables. As to the annual volume of key food commodities, this short tabulation speaks for itself:

Meat products	10,500,000 tons
Cereal products	15,000,000 tons
Vegetables	20,000,000 tons
Fruit	14,000,000 tons
Milk	62,000,000 tons
Dairy products	28,000,000 tons

The manpower requirements are these:

Farm workers	5,000,000
Food processing workers	2,000,000
Food handling and distribution workers	6,000,000
Total	13,000,000

In areas of chaos, the effective control of food distribution could at present only be accomplished initially by the military. In time of peace, military control may come as a disagreeable prospect, to military personnel as well as to civilians. In time of war, it may still be disagreeable but its paramount necessity will quickly make that a secondary consideration.

One of the things we ought to know, but do not, is what happens to people who are struck by great disasters and live through them. Is there a system, a method, we can devise which will help us in handling these people?

Recently a professor at McGill University has actually begun making field studies with the hope of establishing workable rules applicable under the circumstances. He has studied the people affected by several disasters and some knowledge of disaster impact is already accumulated. It appears that only twelve to twenty percent of those who survive a disaster have acted throughout the experience in a somewhat collected manner.

Let me quote Colonel Joseph I. Greene, Editor of the Infantry Journal, on this subject. He says:

"The big point in both combat and disaster, is to find out more about the twelve to twenty percent who keep their heads. A way must be found that will enable us to pick these people in advance. Also, ways must be found to increase this small fraction, which it should be possible to do. For these are the potential leaders and fighters. Upon them the outcome of war (and disasters) will depend."

In maintaining and controlling the food supply, top priority will again have to be given to food for military use at home and overseas. The allocation of food for military rations, processing that food to meet military requirements, and getting that food to the regions, areas, or localities where it is needed are the basic problems. In regard to the processing requirements for military subsistence, we can predict that these should

FIG. 2. Food in profusion and confusion, both. An attempt is being made to salvage and use this food which had been "stored" in the hit-or-miss manner which sometimes forced itself into military operations. Japanese laborers are loading the cans preparatory to transporting them to Japanese charity hospitals. The scene is Tokyo early in 1946. Two American Army Sergeants of the Quartermaster Corps are supervising the job. (Photo No. "Signal Corps 244120.")

not be insurmountable. Except for protecting foods against damage by new types of gas and explosives, our advances in food science and technology are, I think, abreast of the basic needs.

The subsistence problems which may face the Quartermaster Corps in the future may be further described as short-range and long-range problems. An example of a short-range problem, is the fact that the Research and Development Board in its strategic guidance to the Department of Defense has de-emphasized the importance of research in the food field, lowering its priority rating somewhat. The immediate effect of this will be dropping several projects and providing less money for over-all subsistence research for the Department.

An example of a long-range problem is this: In case of destructive attack by air, what kind of emergency organization tells us how to feed great masses of people in chaos areas while civilian authorities are getting set to do the job?

In the event that our food centers, such as Omaha, or Chicago, or Kansas City are wiped out by attack, can the problem of processing and packing of food be accomplished at less vulnerable points? Is the capacity of Industry sufficient to take care of essential needs from these smaller localities?

There was a demand in World War II for packing of certain rations in the square or sardine-type can in order that soldiers could carry these more conveniently in their pockets while fighting in advanced areas. Investigations made at that time showed that it would be necessary to convert Industry at an expense of some twenty million dollars for machinery to produce the cans in sufficient volume to meet these special needs. The task could not be accomplished, not only because of delay in processing, but because the necessary machinery could not be produced due to higher priorities for other instrumentalities of war. This problem may have to be solved in future. In Korea, the soldiers are still asking for a can of this shape.

One problem in which I know you are all interested is the extent to which atomic particles may contaminate a food supply. We do not know the answer, although at the Bikini atomic tests the Army had a considerable quantity of canned foods in the area covered by the explosion. When we got these food cases back to the States, the Geiger-counter showed no radio-activity and when we fed the contents of the cans to rats, there were no adverse effects on these animals.

Another problem is: What effect can bacteriological warfare have on our food stuffs growing in the fields? What effect can the spreading of disease, such as Hoof and Mouth Disease, have on our stock on the ranges and on the farms? In brief, these problems all point up the essential one, that is, whether it is possible through bacteriological warfare to starve a country into submission by enemy Fifth Column or aerial delivery of such contaminating agents.

I happened to be on duty in England prior to our entry into World War II and was attached to the American Embassy. One of the matters in which I was interested was the food stocks which were on hand in the British Isles. From information made public subsequently, during the period from January to April 1941, they were down to 13.8 weeks' supply of flour and 3.3 weeks' of meat. I wonder how we might feel if we were down that low in food stuffs, particularly down to 3 weeks' meat supply? We have no colonies who would be interested in seeing that we were adequately supplied.

I think everyone realizes that the American people could, in an emergency, do with much less food. While we believe in a higher standard of living and high nutritional standards, if we faced a real shortage of food over a protracted period, how low would we be able to go in rationing the people? Great Britain was on a low

FIG. 3. This touching photograph has become known simply as "The Hungry Soldier." He is Pvt. Fred T. Huff, Athens, Ga., one of the soldiers in an American Infantry battalion trapped behind German lines for six days in the Belmont sector of France. He is seen waiting for transportation to the rear, where he can get some rest, and more food. (Photo No. "Signal Corps 196052-S.")

rations scale for a number of years. Many specialists in the field believe the scale was too low. Others tell us that British health conditions were the best in history. The minimum below which any nation can go without causing rebellion of the people has not been established. We ought to know what the minimum scale is.

Some time ago the Army received a recommendation that rations for use by troops be packed separately, by meals, so that all elements necessary for breakfast would be in one case, for dinner in another case, or for supper in a third case. This, of course, would mean that everything from the breakfast food to the bacon involved in a breakfast would have to be assembled in one case. While this would immensely simplify issuing of all rations in the field, it would mean a major assem-

bly job, and the shipping of ration components from all different parts of the country into one central point, repacking them in other cases, and fitting different size cans or containers into a single pack.

This project died when it was pointed out that it would bog down all the transportation facilities in the country in shipping for assembly, and would take space equivalent to all the aircraft factories in order that the tremendous assembly job could be done. While this project is dead for the present, it may arise again as something to be faced.

Experts in transportation and handling have recommended that items shipped overseas be packed on wooden or metal pallets, strapped to such pallets and shipped palletized, by truck, railroad, vessel, or otherwise, as cargo. When you have to store these heavy loads in open fields without materials handling equipment such as fork-lift trucks, which are handicapped in outside storage work, or without cranes to lift heavy loads off the trucks, shipping on pallets seems impractical, particularly in unloading supplies over beaches. Furthermore, according to a survey we recently made, many food processors are not equipped to palletize supplies at the point of manufacture.

FIG. 4. Cpl. Daniel McCarthy, New York City, member of a Quartermaster Corps Test Team, heats up a can of rations for supper while on bivouac in the Mt. Washington, N. H. region. When conditions make group feeding impossible, small individual heaters are used in preparing a soldier's food. (Photo No. RD 3048. Office of the Quartermaster General, U. S. Army, Military Planning Division.)

This, then, is another problem unsolved at present, but which will also arise again. It may be possible, with improved methods, to handle palletized supplies in the field at some future time.

Warfare in the future will involve a great deal of supply by air. Parachute material may be so scarce that it will be impossible to drop all supplies by the parachute method. Further, parachute drop of supplies is uncertain as the wind may carry these parachutes far away from the objective and comparatively small quantities of the total supplies dropped my be recovered by a Force in a small area, particularly when surrounded by the enemy.

The problem here is to find some way we can drop containers by free-fall, either by putting a small attachment on them so that their rate of descent will be slowed down, or by some other method. Maybe the principle of putting on little spinner attachments which will make the case descend like a falling leaf can be applied. The robot plane may find a job for itself here.

It is entirely possible that in case of future war there will not be enough tin for canning food products in the manner to which we are accustomed. What is the answer to this problem? Is it possible, with the present state of technology, to put up food in plastics, procoated cans without tin, or in glass, and still have deterioration impeded to a sufficient extent, or excessive breakage avoided?

We must protect canned supplies and get them to the soldier in usable condition. Rust is accelerated by unusual conditions of transport and unsuitable storage, much of it in the open. During World War II we developed and sent overseas cleaning and coating equipment which made possible the salvage of much food which would otherwise have been lost, but for the future we must find can coatings that remain intact throughout the long military pipeline when exposed to extremes of humidity or temperature.

To revert to the civilian food picture: A possible food shortage, due to enemy destructiveness, raises the question of what we should do about food stockpiling to take care of such contingencies. Should such supplies be stored underground? Someone, either Civil Defense authorities, when these are created, the Army, the Department of Agriculture, or some other agency, may face the problem as to what to do about stockpiling.

We all recall that ocean shipping space was at a premium in the early stages of World War II. Food stuffs comprised a large percentage of the tonnage. Aside from petroleum products, food was probably the major consumer of shipping space. The problem may well arise as to whether we can pack food stuffs into smaller spaces. But how to do this, whether by reshaping containers, or whether we must not only dehydrate food stuffs, but compress them.

We may well find future wars taking place globally, which means under all climatic conditions. It is a moot question whether we should attempt to process and pack food stuffs to meet any climatic condition which may be encountered from the Arctic to the Tropics, or whether we should process foods to meet conditions that may be encountered in the Arctic, and process foods in different containers to meet conditions which might be encountered in Tropical and Desert areas. At present the Army is attempting to develop foods and containers to meet *any* climate conditions. At the moment, this seems the right approach.

There has always been the question whether the Military Service did not over-emphasize the importance of food in quantities to which civilians are accustomed. True, the food we eat forms the energy with which we must accomplish all our tasks. For example, the food we purchase each day in the Military Service has the equivalent in energy of 48 million 30-caliber rifle bullets.

Under some conditions in a campaign, it is impossible to get the accustomed quantities of food to troops. We also know that prisoners in Japan were fed food stuffs on which we had believed it was impossible for them to exist. In many cases this has left no permanent ill effects.

I have long felt that in military maneuvers, instead of bending all efforts to feed the soldier what he has been accustomed to in garrison, we should see on how little he can exist for short periods of time without adversely affecting his efficiency. In some possible critical battle situation, such knowledge might save thousands of lives. The American soldier has the finest ration in the world. Would he fight just as well if we gave him less food? The British soldier, as you know, receives far less food than our soldier does. This problem of cutting the ration may well arise in any future war due to destruction of food stuffs or processing plants.

A noted historian reminds us that the history of the world is the record of man in quest of his daily bread and butter.

This is true. It falls to us, then, as planners of our nation's security to see to it that the particular page of history, dealing with our food supply, shall not be blotted by lack of foresight.

The stock of the world's usable knowledge is small indeed compared with what we have yet to uncover. Only relentless research and realistic tests are capable of adding to the information we need. Sir Isaac Newton, for example, realized this fact. He said: "If I have ever made any valuable discoveries, it has been owing more to patient attention, than to any other talent."

"Patient attention." Simple words. Perhaps they are a summary of whatever upward progress mankind has ever made.

But we do not know how much time we have. We cannot discount the sense of urgency, the elements of national danger, and the need for rapid forward motion toward correct answers. However, slip-shod solutions, which are not solutions at all, these the Army can and will avoid.

The Bacterial Flora of Fresh Pork Sausage[a,b]

WILLIAM L. SULZBACHER AND RUTH A. McLEAN

Bureau of Animal Industry, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland

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The taxonomic distribution of the bacteria found in commercially produced fresh pork sausage was studied. Seventy-five percent of the organisms isolated were members of six species and findings indicated that early deterioration of sausage flavor was the result of acid production by a species of Microbacterium.

The student of meat bacteriology has difficulty in finding references in the literature to the specific microorganisms likely to be found in any particular type of product, especially fresh pork sausage. Some older studies touch briefly on this subject, such as the very good paper by Carey *(2)* in 1916, but most of the published work deals only with quantitative studies of the bacterial population. Even in this respect the work was greatly hampered by the difficulty of obtaining meat suspensions for analysis when one had to resort to such methods as grinding with sand or shaking with broken glass. There is, therefore, urgent need for more up-to-date investigations.

During the last two years we have been engaged in a study of the effect of processing factors on the microbiological spoilage of fresh pork sausage. In the course of this study, which is still in progress, we have had the opportunity of studying a fairly large number of bacterial cultures isolated from this product. This paper deals with the taxonomic distribution of most of the organisms isolated and also describes, briefly, the technique used for sampling.

[a] This paper includes material presented by the authors at the 1950 annual meeting of the Society of American Bacteriologists.

[b] The authors acknowledge, with thanks, the valuable assistance of Mrs. Nina B. Hess in conducting the laboratory investigations.

METHODS EMPLOYED

Samples were collected in commercial sausage plants at the time of manufacture. The material sampled, either finished sausage or material collected at different stages of preparation, was placed in sterile friction top cans and packed in crushed ice for transportation to the laboratory. Most of the samples were collected during the early morning and plates were poured by noon of the same day, although in a few instances, owing to the large number of samples involved, plating was not completed until 1 or 2 o'clock. The present report includes material on cultures isolated from samples collected in three commercial establishments. All three were fairly large producers and their product is representative of the type of fresh pork sausage to be found on the market in any Eastern metropolitan area.

In the preparation of the dilutions for plating, 50 g. of sausage were weighed into a sterile, tared Waring Blendor jar and mixed for 1 minute with 150 ml. of physiological salt solution to give a dilution of 1 part in 4. Four g. of this mixture were then added to a sterile, tared Erlenmeyer flask by dropping the mixture from a large-bore, straight-sided pipette, and 96 ml. of saline added to give a 1 to 100 dilution. From this flask further decimal dilutions were made by pipetting in the usual manner.

Aerobic and anaerobic counts were made using agar prepared by adding 1.5 percent agar and 0.5 percent salt to Difco veal infusion medium. Aerobic plates were incubated at 20° C. and anaerobic plates at 37° C. in an anaerobic jar.

Lipase-forming organisms were determined by plate counts using a modification of the Jensen-Turner *(4)* method. The medium used consisted of 250 ml. of the veal infusion agar described above to which were added 2.5 g. of lard and 37 ml. of a freshly filtered 0.1 percent aqueous solution of Nile Blue A. This mixture was autoclaved and while still hot was mixed for 1 minute in a sterile Waring Blendor jar. The agar was poured directly from the blendor jar as soon as it was sufficiently cool and the plates were incubated at 20° C. The appearance of colonies is the same as that described by Jensen.

All samples were tested for the presence of members of the genus *Salmonella* by inoculating tubes of tetrathionate broth with portions of the sausage suspensions and streaking on S S

agar after 18 hours' incubation. Suspected colonies were transferred to slants of Kligler's iron agar for further study.

The plates intended for counting were inspected carefully and transfers to agar slants were made from all colonies showing any macroscopic difference. Slants were inoculated from the various colonies in approximate proportion to the prevalence of the colony type, so that the number of isolates later studied bore a rough relationship to the distribution of the respective organisms in sausage. Some isolations were also made from spot plates used to determine the extent of contamination on the various pieces of plant equipment.

Included in this study were organisms isolated from spices. The spice samples were collected in the sausage plants visited and dilutions were prepared for plating by mixing weighed quantities of spice with physiological salt solution in a Waring Blendor and following the same general procedure as that outlined for sausage.

DISTRIBUTION OF ORGANISMS

Table 1 shows the distribution, according to genera, of organisms isolated arranged in the order in which they occur in Bergey's Manual *(1)*. As mentioned above, this distribution roughly approximates the distribution in sausage. However, the 21 *Proteus* isolates are not typical as to prevalence because they were all isolated from S S agar which had been streaked from tetrathionate enrichment broth and they must actually comprise far less than 6.7 percent of the normal sausage flora. The four cultures of *Xanthomonas* were all isolated from spice samples and were culturally quite similar to *Xanthomonas oryzae*, a rice pathogen.

TABLE 1

Generic Distribution of Organisms Isolated From Pork Sausage

Genus	Number of Isolates	Percent of Total
Pseudomonas	34	10.8
Xanthomonas	4	1.1
Micrococcus	3	1.0
Sarcina	5	1.6
Neisseria	1	0.3
Microbacterium	47	14.9
Alcaligenes	19	6.0
Achromobacter	40	12.7
Flavobacterium	8	2.5
Escherichia	2	0.6
Paracolobactrum	2	0.6
Aerobacter	2	0.6
Proteus	21	6.7
Bacterium	65	20.6
Bacillus	28	8.9
Misc. unidentified	28	8.9
Yeasts	7	2.2
Total	316	100.0

More than 74 percent of the isolates belong in six genera. These are shown in Table 2 together with a key to their relative prevalence in either freshly prepared sausage, older samples stored in the refrigerator, spices, or plant equipment.

Seventy percent of the *Pseudomonas* cultures studied were found as lipase-forming organisms in new sausage.

TABLE 2

Habitat Distribution of Bacteria Isolated

Genus	Number of Isolates	Percent of Total	New Sausage	Old Sausage	Spices	Equipment
Pseudomonas	34	10.8	+ + +	—	—	+
Microbacterium	47	14.9	+ +	+ + + +	—	—
Alcaligenes	19	6.0	+	+ +	—	—
Achromobacter	40	12.9	+ +	+ +	—	+
Bacterium	65	20.6	+ + +	+ +	—	+
Bacillus	28	8.9	+	+	+ +	+
Total	233	74.1				

Although many of these organisms were also proteolytic, they evidently do not contribute greatly to the deterioration of the sausage because they were not recovered in significant numbers from the stored samples.

Species of *Microbacterium* made up a rather large proportion of the flora of fresh pork sausage which had been stored at home refrigerator temperatures (5-8° C.) and may have been responsible for the acid flavor of these samples. All 47 isolates recorded in Table 2 were evidently members of a single species. Although they differ from the described species of *Microbacterium (1)* in that they are not heat resistant, they are undoubtedly members of this genus. All 47 were Gram positive, nonsporeforming bacilli that produced lactic acid and CO_2 from carbohydrates. All were catalase positive, non-motile and did not liquefy gelatin or reduce nitrates to nitrites. A study of their fermenting powers showed that they produced a mixture of lactic acid isomers. The average specific rotation obtained for the zinc lactate, at a concentration of 3.5 g. per 100 ml. was $[\alpha]_D^{28} = -6.2°$. It is also interesting to note that Niven and coworkers *(5)*, and others have implicated lactobacilli in spoilage studies of other types of sausage.

The *Alcaligenes* species isolated were slightly more prevalent in stored sausage samples while *Achromobacter* and *Bacterium* species were more generally found in freshly prepared sausage although they are not particularly selective as to habitat. Members of the genus *Bacillus* were evenly distributed in all the types of environment studied except that, as might be expected, they made up the bulk of the flora found in spices. Nineteen of the 28 cultures isolated were *B. subtilis*.

Careful examination of the tetrathionate enrichment cultures failed to show any truly aglutinable strains of *Salmonella*. A few closely related forms were isolated but none that showed typical *Salmonella* aglutinations. This finding is in agreement with the work of Goettler *(3)*.

SUMMARY

Cultures of three hundred and sixteen microorganisms have been isolated from fresh pork sausage. They have been classified according to genera and a rough approximation has been made of their prevalence and of their persistence during storage. Members of the genus *Microbacterium* may be responsible for the development of the acid taste in stored sausage.

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Application of Balanced Incomplete Block Design to Scoring of Ten Dried Egg Samples[a]

HELEN L. HANSON, LEO KLINE, AND HANS LINEWEAVER
Western Regional Research Laboratory,[b] Albany, California

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The usefulness of a balanced incomplete block design in judging of flavor differences is described for conditions when the number of samples to be compared is larger than can be judged reliably at one time. The design provides for efficiency of time and material, elimination of judges' fatigue, and maintenance of judges' interest. The method is illustrated by results obtained in judging 10 samples of dried eggs. The marked effect of the quality accompanying samples on scores given to a particular sample is also shown.

One of the limitations of food flavor evaluation methods is the uncertainty encountered in the comparative judging of more than four to six samples at one sitting. The uncertainty arises, of course, from reduced acuity, as well as from confusion or fatigue associated with making a large number of comparative judgments at one time. However, it usually happens that an experiment designed to test only two or three variables results in as many as 10 or 12 distinct samples, with direct comparisons needed between all samples. Such a situation was encountered in studying the deteriorative reactions that occur in dried whole eggs at an elevated temperature. The experiment was designed to test the effect of three variables: (1) Moisture content of the egg powder, (2) gaseous atmosphere in which the powders were stored, and (3) glucose removal before drying. A determination was needed of the relative off flavor development in ten samples of dried egg, differing from each other in the three variables listed.

In selecting the method used to obtain the information required, consideration was given to the amount of time and material required by various designs, the number of samples to be judged at one time, the possibility of direct comparison of results from all ten samples, and the elimination of prejudice that would result from knowledge of which of the ten samples would be judged on any day.

Of the possible methods available, the most expeditious from the point of view of the time required is that of judging all ten samples at a time. Obviously a rigid upper limit cannot be placed on the number of samples that should be tasted at one sitting, since the number is influenced by such factors as the accuracy required, the strength of flavor or off flavor of the samples, and the ability of the judges. The method was not used because it was believed that the psychological effect on the judges, if not the actual fatigue, caused by requiring comparisons of ten samples would result in loss of interest and poor performance.

An alternative method is to divide the total number of samples into several smaller groups and to compare each group with a standard or control sample. In spite of the use of the control, such a method provides a reliable comparison only for the samples actually compared at the same time. Thus, a treatment in one group receiving a score of 6 may or may not have the same flavor as a treatment in another group receiving that same score, because the score a sample receives has been found to depend to an important extent on the quality of the other samples judged at the same time. Illustrative data will be presented on this point.

Another method is to make a separate comparison between every two treatments being investigated. With sufficient replications such a method will provide all of the information required about differences in flavor between any number of treatments. However, in a situation where ten treatments must be compared, sufficient replication for statistical analysis of the results from every possible pair of treatments requires an amount of time and material that is seldom available. A separate comparison of every two of the ten treatments tested in the dried egg experiment would have required 45 judging periods, 9 replications of each sample, and a total of 90 samples tasted for only a single comparison of each pair of treatments.

The method selected for this experiment was the statistical plan known as the balanced incomplete block design, a design that had been found useful in work with biological samples. In this design only a part of the total number of samples are judged at the same time. Each sample is compared with every other sample equally often. Designs are available for any total number of samples and for any fraction of the total number to be compared at one time *(1)*. Since only a few of the samples are compared at any one time, the design minimizes fatigue and acuity errors, yet it permits more reliable comparisons between samples from large numbers of treatments than is obtained by comparison of samples with controls and requires less tasting than is required when every pair of samples is tested separately (15 judging periods, 6 replications, and a total of 60 samples tasted were required in the balanced incomplete block design used). The design has been used in this laboratory in more than 12 experiments on products of such varying nature as dried eggs, turkey steaks, roast turkey, and peas.

Independently, Galinat and Everett *(3)* at the Connecticut Agricultural Experiment Station used a balanced incomplete block design to determine consumer preference for sweet corn. These authors were interested in arranging nine varieties of corn in order of preference, but they were aware of the psychological difficulties involved in ranking nine samples at one time. Therefore, the samples were presented three at a time. Members of the Experiment Station staff were asked to rank the samples in each set in order of preference. On the basis of results obtained, the authors felt that they could eliminate the lower-ranking samples and could limit a supplementary large-scale consumer test to the two or three samples that received the highest preference ranking by the small panel.

[a] Presented at the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 24, 1950.

[b] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study in which certain phases were carried on under the Research and Marketing Act of 1946.

The principal purpose of this paper is to describe the usefulness of the balanced incomplete block design as illustrated by the dried egg experiment. The results, which were obtained in terms of scores rather than ranks, will be analyzed and compared with other data in order to provide support for the validity of results and comparisons obtained with the design.

MATERIALS AND METHODS

The preparation of the dried whole egg samples and the chemistry of their deterioration will be described in other publications from this Laboratory. It is sufficient here to list the ten samples.

Sample No.	Treatment
1.	Control (Normal), 2% moisture, air pack, —30° F. (—34° C.)
2.	Control (Glucose-free), 2% moisture, air pack, —30° F. (—34° C.)
3.	Glucose-free powder, 2% moisture, N_2 pack, 100° F. (37.8° C.)
4.	Glucose-free powder, 2% moisture, air pack, 100° F. (37.8° C.)
5.	Glucose-free powder, 5% moisture, N_2 pack, 100° F. (37.8° C.)
6.	Glucose-free powder, 5% moisture, air pack, 100° F. (37.8° C.)
7.	Normal powder, 2% moisture, N_2 pack, 100° F. (37.8° C.)
8.	Normal powder, 2% moisture, air pack, 100° F. (37.8° C.)
9.	Normal powder, 5% moisture, N_2 pack, 100° F. (37.8° C.)
10.	Normal powder, 5% moisture, air pack, 100° F. (37.8° C.)

In the design selected for the taste judging, samples from the ten treatments were set up in fifteen combinations of four samples each. Each set or block of four was tasted once. Each sample was tasted six times and each pair of samples appeared together twice. The standard design used *(1)* was as follows:

Block	Samples	Block	Samples	Block	Samples
(1)	1 2 3 4	(6)	1 6 8 10	(11)	3 5 9 10
(2)	1 2 5 6	(7)	2 3 6 9	(12)	3 6 7 10
(3)	1 3 7 8	(8)	2 4 7 10	(13)	3 4 5 8
(4)	1 4 9 10	(9)	2 5 8 10	(14)	4 5 6 7
(5)	1 5 7 9	(10)	2 7 8 9	(15)	4 6 8 9

For complete randomization the order of judging the blocks of four was randomized, the order of tasting the four samples within each block was randomized and the treatments were randomly assigned to the sample numbers in the design. However, to simplify reporting of the results, the sample numbers given in this paper are the original numbers listed with their corresponding treatments at the beginning of this section.

In preparation for judging the degree of storage off-flavor, the dried eggs were reconstituted and cooked with stirring on a steam bath until they were sufficiently coagulated. The scrambled eggs were served to the panel of seven judges under sodium vapor lights to mask color differences. The color differences were successfully masked in all cases except those in which the powders showed extreme browning at the 3 and 6 months storage periods.

RESULTS AND DISCUSSION

The Influence of Quality of Other Samples in a Block on the Score Assigned to a Given Sample

When the balanced incomplete block design was selected for use, it was anticipated that the scores given to any one sample would be influenced by the quality of the other samples judged at the same time. Such an effect has been reported by Dove *(2)* and Hopkins *(4)*. The magnitude of the effect was surprisingly large in the dried egg experiment. This result, of course, is added evidence that scores are relative rather than absolute. Table 1 shows the degree to which a panel may be influenced by the quality of samples judged at the same time. The ten samples from left to right show increasing off-flavor. As would be expected, the greatest range in scores was found in the samples of intermediate quality. These samples were given high

TABLE 1

Panel Means Showing Influence of Quality of Other Samples on Scoring Level [c]

Judging period	Sample number									
	1 (control)	2 (control)	3	4	5	6	7	8	9	10
1	9.7	8.7	....	5.4	5.0	....	....	....	....	....
2	....	9.6	8.8	....	....	5.6	....	....	....	3.6
3	....	9.0	....	7.3	....	3.8	4.3	....	....	....
4	9.3	....	8.7	....	6.8	....	3.8	....	....	....
5	10.00	....	....	7.5	....	....	....	4.2	....	2.8
6	....	9.6	....	....	....	....	5.1	4.6	3.6	....
7	....	9.8	....	....	7.4	....	....	4.4	....	3.8
8	....	....	....	....	9.4	....	6.3	....	5.1	2.0
9	9.3	9.3	8.2	....	....	....	....	....	3.3	....
10	....	....	....	8.7	9.0	6.0	....	....	3.3	....
11	9.7	....	....	....	....	6.7	6.6	....	....	2.8
12	....	....	9.3	8.1	....	....	....	....	3.7	2.6
13	9.8	....	....	....	....	7.3	....	5.4	4.0	....
14	....	....	9.0	8.3	....	....	4.8	3.8	....	....
15	....	....	9.3	....	8.3	6.3	....	3.8	....	....
Mean	9.7	9.3	8.9	7.6	7.7	6.0	5.3	4.5	3.9	3.0
Range	0.7	1.1	1.1	3.3	4.4	3.5	2.8	1.6	1.8	1.8

[c] 10 = No off-flavor; 0 = extreme off-flavor.

scores when scored at the same time as samples having greater degrees of off-flavor and were given low scores when scored at the same time as samples having lesser degrees of off-flavor. Sample Number 5, for example, shows a variation in score from 5.0 to 9.4. It received a score of 5.0 when there was no sample present having a greater degree of off-flavor and when both controls were present. It received a score of 9.4 when it had the least off-flavor of any of the samples being graded at that period and when the worst of the ten samples was present. Also, for the two times that two controls were present (judging periods 1 and 9) the other samples graded at those two periods received the lowest scores they were ever given. But when no control was present (judging periods 8, 10, 12, 14, and 15), the samples having the least off-flavor that were present at those periods (Sample 5 with a score of 9.4, Sample 4 with 8.7, and Sample 3 with scores of 9.3, 9.0 and 9.3) received the highest scores they were ever given. The results obtained with this same design after 3 months' storage of the powders show a similar effect of the quality of the samples present on the scores.

Only five samples were judged at the 6-month storage period because the off-flavor development in some of the other samples was so great that no purpose would be served by scoring them. The scores obtained on these five treatments, which were judged four at a time, also showed that the panel was affected by the quality of the samples present in the block being scored, but the scores differed to a considerably less degree than is shown in Table 1. Since only one of the five treatments was omitted at each judging period, less fluctuation in the scores would be expected.

From the results of Table 1 it is evident that the error of the panel means for the intermediate samples is greater than that of the other samples. Fortunately, the balance provided in the design by the requirement that every sample be judged with every other sample an equal number of times tends to ensure that all samples will be judged under an approximately equivalent series of conditions when all blocks are considered to-

gether. Since the statistical analysis of results is based on the seven individual judge's means for each sample (Table 3) rather than on the six panel means (Table 1) these fluctuations in scores of the samples of intermediate quality do not enter into the analysis directly. However, it would be very desirable to decrease the range of scores assigned to these samples.

Since the judges are all affected in the same way by the quality of samples present, the variation in scores is not of a random type and therefore should be reduced by the use of appropriate standards. Provision for a labeled control having no off-flavor in every judging period would be of value in preventing the tendency of judges to grade the better experimental samples higher in the absence of a control than in the presence of a control. The results of this experiment (Table 2) show

TABLE 2

Influence of Unlabeled Control on Scores Given Samples of Varying Degrees of Off-Flavor (From Table 1)

Sample No.	Scores in presence of one control	Scores in absence of any control
3	8.8, 8.7	9.3, 9.0, 9.3
4	7.3, 7.5	8.7, 8.1, 8.3
5	6.8, 7.4	9.4, 9.0, 8.3
6	5.6, 3.8, 6.7, 7.3	6.0, 6.3
7	4.3, 3.8, 5.1, 6.6	6.3, 4.8

that the presence of an unlabeled control had its greatest effect on the scores of the samples having relatively little off-flavor. The scores assigned to Samples 3, 4, and 5 were strongly influenced by the presence or absence of a control, but the scores assigned to samples with more off-flavor than was present in Sample 5 were only slightly different in the presence and absence of a control. Undoubtedly the use of one or two labeled standards of intermediate quality would stabilize the scores assigned to unknowns since the standards would serve as reference points for the judges. Such standards should aid in stabilizing the scores for experimental samples of intermediate quality. Such standards could be used to the best advantage when the type and intensity of off-flavor to be encountered is known to the investigator. Where a homogeneous product is being investigated it might be advantageous to mix the best and worst samples in suitable proportion to give an adequate intermediate standard.

Consistency of Results Obtained by Use of Balanced Incomplete Block Design

In spite of the day-to-day fluctuations in panel means related to the quality of the samples judged at one time, the final results obtained were consistent. The following section shows that comparable results were obtained by the individual judges; that similar results were obtained at three different storage periods; that results obtained by the balanced incomplete block design agreed with those obtained by a randomized complete block design; and that differences between any two samples in the two replications where they were judged side by side were similar to the differences between their final scores after all six replications.

In the following discussion statistical significance of differences refers to the significance obtained by analyzing flavor scores by the method of analysis of variance, using the individual judge's means for each sample as the replicate determination rather than the panel means. The data have also been treated by other methods, but this paper will report only the results of the above described treatment, which was suggested by Lyle D. Calvin of the Institute of Statistics of the University of North Carolina.*

Table 3, containing the mean scores for each of the seven judges for off-flavor of the ten samples, shows

TABLE 3

Judges' Mean Scores for Ten Samples of Egg Powder After One-Month Storage at 100° F. (37.8° C.)

Judge	Sample number									
	1	2	3	4	5	6	7	8	9	10
R F	10.00	8.75	9.20	7.60	8.67	6.60	5.20	4.67	5.80	3.50
J G	9.83	9.83	8.83	7.83	6.83	5.00	4.83	4.33	3.67	2.33
H H	9.67	9.00	8.83	7.17	7.50	5.83	5.17	4.00	3.67	2.00
L K	9.80	10.00	8.60	7.50	8.60	5.83	5.80	3.00	3.00	3.00
A K	8.80	9.17	8.67	7.40	7.33	5.83	5.33	5.00	5.17	4.20
H M	9.83	9.50	9.25	8.20	7.00	6.40	6.20	6.00	2.33	3.33
R R	9.67	9.00	9.00	7.33	7.83	6.67	4.33	4.50	3.67	2.50
Mean	9.66	9.32	8.91	7.58	7.68	6.02	5.26	4.50	3.90	2.98
Range	1.20	1.25	0.65	1.03	1.84	1.67	1.87	3.00	3.47	2.20

close agreement among the judges, particularly for the seven samples of highest quality. The greater variation in scoring of the three poorest samples is probably related to the fact that the samples had different ***kinds*** of off-flavor rather than different degrees of one kind of off-flavor. The air-packed samples (8 and 10) developed a "fishy" off-flavor, and the nitrogen-packed sample (9) developed a "cardboard" off-flavor. The judges did not always agree on which of these off-flavors should receive the lower score.

Table 4 shows that the samples were placed in the same order with respect to degree of off-flavor at each

TABLE 4

Means Showing Comparable Results at Different Storage Periods

Sample No.	Storage time at 100° F. (37.8° C.)		
	1 Month	3 Months	6 Months[a]
1	9.7	9.6	9.7
2	9.3	9.4	....
3	8.9	8.0	6.6
4	7.6	6.0	4.0
5	7.7	5.9	....
6	6.0	4.1	....
7	5.3	3.0	2.7
8	4.5	3.0	2.0
9	3.9	2.4	....
10	3.0	1.4	....

[a] Only five samples were judged at the 6-month storage period. The five samples were judged four at a time. Each sample was judged four times and each pair appeared together three times.

of the three storage periods. Samples 1 and 2 and Samples 4 and 5 were not significantly different from each other at either storage period. Sample 7 was graded unexpectedly low in relation to 8 at the 3-month period, probably due to the fact that the lights failed to mask its darker color and the panel was unable to score the flavor without being influenced by the color. After six months of storage both of the samples were dark.

Four of the ten treatments tested in this experiment were also evaluated in a separate experiment using a

*Private communication.

TABLE 5

Comparison of Scores[a] With Balanced Incomplete Block Design and Randomized Complete Block Design

Statistical design	Storage time at 100° F. (37.8° C.), weeks	Sample number			
		1	2	4	8
Randomized Complete Block	4	9.6	9.6	7.5	4.5
Balanced Incomplete Block	4	9.7	9.3	7.6	4.5
Randomized Complete Block	10	9.6	9.7	6.5	3.9
Balanced Incomplete Block	12	9.6	9.4	6.0	3.0

[a] 10 = no off-flavor. 0 = extreme off-flavor.

randomized complete block design. Table 5 shows the results of evaluation of Samples 1, 2, 4, and 8 after 4 and 10 weeks' storage by a randomized complete block design and after 4 and 12 weeks' storage by the balanced incomplete block design. The remarkable consistency in scores obtained in the two experiments was probably fortuitous. In all cases, however, the differences between the two experimental samples and between the experimental samples and the controls were highly significant ($P = 0.01$). Evidence that essentially the same results were obtained with both experimental designs indicates that for these treatments at least no information was lost by use of the incomplete block design.

As previously noted for the balanced incomplete block design used in this experiment, there were two direct comparisons between every pair of samples. It is reasonable to suppose that the relative off-flavors can be judged more precisely when the samples are judged at the same time, than when they are judged with different samples in different blocks. In order to determine whether this was true with the present data, a comparison of results for all six replicates with the two replicates for the same samples side by side in one block was made. In both instances the differences between a series of pairs are reported (Table 6). The differences shown for the means of six replicates were all statistically significant and are confirmed in every case by the side by side comparisons. This confirmation of mean scores has been found in other experiments using a balanced incomplete block design and is evidence of the validity of the results obtained within any one experiment.

Information Obtained on Relative Effect of Glucose Removal, Atmosphere, and Moisture Content Under All Experimental Conditions

The results in Table 7 show the effect of glucose removal under four different conditions of storage atmosphere and moisture content of the powder; they show the effect of storage atmosphere under four conditions of moisture and glucose content; and they show the effect of moisture content under four conditions of atmosphere and glucose content. In addition, they show that, of the three variables studied, after storage at 100° F. (37.8° C.) for four weeks, the greatest effect on storage stability of the powder was obtained by removal of glucose from the egg before drying. Comparable results were obtained after storage of the powders for three and six months.

These results indicate that the design used provided reliable comparisons of the treatments imposed. Comparisons with the results of rather extensive chemical analyses of these samples, to be reported elsewhere, also give strong support to the reliability of the results obtained with this design.

TABLE 6

Comparison of the Differences Using All Six Replicates With Those for the Two Replicates in Which the Samples are Judged Together in the Same Block

Conditions		Mean differences between glucose-free and normal samples[f]	
Moisture percent	Atmosphere	All six replicates	The two replicates with samples in the same block
2	N_2	3.6	4.6
2	Air	3.1	3.9
5	N_2	3.8	5.0
5	Air	3.0	3.0
Conditions		**Mean differences between N_2 and air storage atmosphere[f]**	
Moisture percent	**Glucose content**	**All six replicates**	**The two replicates with samples in the same block**
2	Glucose-Free	1.3	1.0
5	Glucose-Free	1.7	2.5
2	Normal	0.8	0.8
5	Normal	0.9	2.1
Conditions		**Mean differences between 2 percent and 5 percent moisture content[f]**	
Atmosphere	**Glucose content**	**All six replicates**	**The two replicates with samples in the same block**
N_2	Glucose-Free	1.2	1.5
Air	Glucose-Free	1.6	3.1
N_2	Normal	1.4	1.4
Air	Normal	1.5	1.0

[f] In all cases the glucose-free samples, the N_2 packed samples, and those containing 2 percent moisture had the least off-flavor.

TABLE 7

Effect of Glucose Removal, Storage Atmosphere, and Moisture Content on Storage Stability of Samples After One-Month Storage at 100° F. (37.8° C.)

Condition		Scores showing differences due to glucose removal		
Moisture percent	Atmosphere	Glucose-free	Normal	Difference
2	N_2	8.9	5.3	3.6[a]
2	Air	7.6	4.5	3.1[a]
5	N_2	7.7	3.9	3.8[a]
5	Air	6.0	3.0	3.0[a]
Condition		**Scores showing differences due to storage atmosphere**		
Moisture percent	**Glucose content**	**N_2**	**Air**	**Difference**
2	Glucose-Free	8.9	7.6	1.3[a]
5	Glucose-Free	7.7	6.0	1.7[a]
2	Normal	5.3	4.5	0.8[b]
5	Normal	3.9	3.0	0.9[b]
Condition		**Scores showing differences due to moisture content**		
Atmosphere	**Glucose content**	**2 percent Moisture**	**5 percent Moisture**	**Difference**
N_2	Glucose-Free	8.9	7.7	1.2[c]
Air	Glucose-Free	7.6	6.0	1.6[a]
N_2	Normal	5.3	3.9	1.4[a]
Air	Normal	4.5	3.0	1.5[a]

[a] Difference significant at 5.1 percent level.
[b] Difference significant at 1 percent level.
[c] Difference significant at 0.1 percent level.

Value of Balanced Incomplete Block Design in Eliminating Prejudice Due to Knowledge of Samples to Be Scored

In addition to its usefulness in cases where the number of samples to be compared is larger than a panel can evaluate at one sitting, the balanced incomplete

block design has a psychological advantage. Because all samples in the experiment are never presented at the same time, the design offers a solution to the problem of giving the judges enough information about the experiment to retain their interest in it without giving them so much information that their judging may be prejudiced. Even though the judges are told at the beginning of a storage experiment for example, what conditions are being investigated, they have no way of predicting which of the treatments will be judged on any one day. It is even possible to use as judges those individuals who have worked closely with the experiment, without having their added knowledge of the treatments prejudice their scoring. Since each judging period brings a different series of samples, the judge is forced to evaluate each set of samples on its merits without mental reference to previous experience in judging the same set. After each day's judging, the panel may be told which samples were evaluated and may compare their results without danger of influencing their future scoring. Individual judges who have scored samples by several procedures are enthusiastic about this feature of the balanced incomplete block design, which permits comparison of scores after each tasting period.

CONCLUSIONS

The balanced incomplete block design is a useful method of judging flavor differences when the number of samples is larger than can be judged reliably at one sitting. Reliability of the method was indicated in the case cited by examining the results obtained within the 10-sample dried egg experiment for consistency and by comparing scores assigned to four of the 10 samples in the balanced incomplete block design with their scores in a complete block design.

For the 10 samples tested the design avoids fatigue and acuity errors that would be encountered if all samples were tasted at one sitting. The design used is more efficient of time and material than one requiring a separate direct comparison of every possible pair of samples. It is superior to a design in which the 10 samples are divided into groups and each group is compared with a control, since it has been shown that direct comparisons between the scores of the different groups are not valid. The design has a psychological advantage in that the judges' interest may be retained by informing them about all samples to be compared, and yet they are not prejudiced since they are unable to determine which samples will be judged on any one day.

An illustration is given of the effect of the quality of other samples judged at the same time on the scores given to samples. The variations illustrate the danger of comparing samples scored at different times even though each sample may have been scored in comparison with a control.

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The Acids of California Grapes and Wines. II. Malic Acid[a]

MAYNARD A. AMERINE
University of California, Davis, California

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Malic acid was determined following removal of tartrates. During ripening there was a relative and absolute decrease in percent acid (or malates). For the 12 California varieties of grapes tested at maturity malic acid ranged from 14 to 45 percent. These figures are substantially in agreement with those reported for varieties in Europe (13 to 47 percent), and appear to be fairly constant for any given variety. On theoretical grounds low malic-acid varieties seem best suited to California climatic conditions.

Malic acid was first identified in grapes by Schwarz *(11)* in 1852 and various authors, including Franzen and Keyssner *(3)*, have confirmed its presence. The primary difficulty in its determination is the presence of tartaric acid, many of whose properties are similar to those of malic acid.

The two procedures apparently most suitable for its determination are those of Pucher, Vickery, and Wakeman *(10)*, and of Peynaud *(8)*. The former avoids interference of tartaric acid but is lengthy. Peynaud's procedure is really a modification of Mezetrat's 1904 technique. Barium malate and tartrate are separated from barium succinate in 65-70 percent alcohol and oxidized to acetaldehyde in a buffered acid solution using dilute permanganate. The aldehyde is collected in a bisulfite solution and titrated by the Jaulmes and Espezel technique. The acetaldehyde resulting from the oxidation of tartaric acid is calculated from a separate determination of the tartrates and subtracted. This subtraction assumes a constant oxidation factor of tartaric acid to acetaldehyde.

As shown in Table 1, it is difficult to obtain a constant oxidation factor in experiments with tartaric and malic acid. Therefore, Amerine and Winkler *(2)* removed most of the tartrates

[a] Presented before the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 22, 1950.

by precipitation as potassium acid tartrate before oxidation of the malic acid. This technique resulted in a fairly uniform recovery of malic acid. A value of 88 percent recovery has been used in our calculations. These results are in substantial agreement with those of Joslyn and Comar *(6)*, who found recovery of acetaldehyde by the Jaulmes and Espezel procedure to range from 78 to 92 percent.

TABLE 1

Influence of Removal of Tartaric Acid on Recovery of Malic Acid

Malic acid	Tartaric acid	Tartrates removed	Recovery malic acid
mg.	*mg.*		*percent*
5	5	No	87.2
5	5	No	93.6
5	5	No	107.0
10	10	No	101.0
10	10	No	112.0
25	25	No	78.8
25	5	Yes	89.4
25	25	Yes	83.4
12.5	12.5	Yes	90.0
25	12.5	Yes	90.0
25	....	....	89.1
10	....	....	88.2

CHANGES DURING MATURATION

The characteristic feature of the ripening of grapes is the simultaneous increase in sugars and decrease in total titratable acidity. Considerable practical importance is attached to these changes, as the utilization of the mature grape depends to a large extent on the percentage of sugar and acids in the fruit at harvest time.

The literature of this topic has been reviewed by Amerine and Winkler *(2)*, Génevois *(4, 5)*, and Peynaud *(9)*. The earliest data are those of Mach in 1879 *(7)*. His analytical procedures may be subject to criticism, yet the general nature of the changes that occur are clearly indicated. More detailed studies are presented by Peynaud *(9)* and Amerine and Winkler *(2)*. These later data indicate the decrease of total malic acid and of malates as percent of the total acid and the increase in the percent of the titratable acidity due to tartrates.

Although our results and those of Peynaud do not differ greatly, the method of securing the samples is not the same, and comparisons of the two procedures should be made. Peynaud extracted the crushed grapes with hot water, then boiled and stored the juice, whereas we have used a hydraulic press after crushing and have made immediate analyses. The disadvantage of Peynaud's technique lies in the nonuniformity of the juice of the different parts of the berry, and in the possible changes in the composition of the juice effected by boiling. The differences between the composition of free-run and press-juice from the same grapes is indicated in Table 2. Probably no system of obtaining a sample is altogether satisfactory from the practical point of view. In some cases in winery practice only the free-run juice is used; in others, the skin, pulp, and free-run juice are all fermented together.

The post-war data obtained at this station are presented in Table 3. The method of presenting this data is similar to that of the pre-war studies in showing the regular and simultaneous decrease in tartaric and malic acids during maturation.

Malates, calculated as percent of the total tartrates and malates actually determined, also decreased. Peynaud *(9)* has found that the decrease is much the same if the data are calculated on a per fruit basis. In the Malbec variety tartrates decreased from 0.20 to 0.15 g. per 100 grape berries between August 10th and September 30th while malates drpped from 0.45 to 0.10 during the same period.

TABLE 2

Acid Content of the Free-run Juice[c] Versus the Press Juice[d]

Variety	Free-run juice		Press juice	
	Tartrates as tartaric	Malates as malic	Tartrates as tartaric	Malates as malic
	percent	*percent*	*percent*	*percent*
Thompson Seedless	0.57	0.17	0.74	0.26
Thompson Seedless	0.45	0.06	0.40	0.08
Malbec	0.51	0.16	0.67	0.15
Malbec	0.47?	0.14	0.63	0.15
Cabernet Sauvignon	0.44	0.14	0.51	0.15
Cabernet Sauvignon	0.40	0.12	0.55	0.10
White Riesling	0.56	0.09	0.56	0.09
White Riesling	0.46	0.16?	0.56	0.15
Semillon	0.48	0.13	0.56	0.18
Semillon	0.48	0.11	0.62	0.13
Carignane	0.65	0.21	0.72	0.17
Carignane	0.56	0.15	0.54	0.10

[c] From a screw-type crusher.
[d] From the residue in a hydraulic press.

VARIETAL DIFFERENCES

Wine grape varieties differ greatly in their suitability for producing the various types of wines. This is partially due to the different amounts of total acidity in the grapes. In the course of our studies on the quality of the wines produced from the several varieties, variations in quality were observed that could not be

TABLE 3

Changes in Composition of Six Important Wine Grape Varieties During Maturation, 1947

Date	Balling, degree	Total acid, percent	pH	Tartrates, as tartaric, percent	Malates, as malic, percent	Malates as percent of total
Cabernet Sauvignon						
Aug. 14	21.2	1.13	3.16	0.58	0.36	38.3
Aug. 27	22.9	0.66	3.49	0.57	0.20	25.9
Sept. 10	26.2	0.62	3.60	0.53	0.17	24.3
Sept. 24	28.5	0.50	3.85	0.44	0.14	24.1
Oct. 6	28.4	0.50	4.01	0.40	0.12	23.1
Carignane						
Aug. 18	15.9	1.29	2.90	0.78	0.50	39.0
Aug. 27	17.2	1.05	3.10	0.77	0.24	23.7
Sept. 12	19.0	0.90	3.11	0.71	0.16?	18.4?
Sept. 26	19.1	0.81	3.28	0.65	0.21	24.4
Oct. 14	21.4	0.70	3.51	0.56	0.15	21.1
Malbec						
Aug. 12	18.9	1.35	3.45	0.48	0.60	55.5
Aug. 26	23.0	0.83	3.70	0.54	0.26	32.5
Sept. 9	24.6	0.77	3.70	0.53	0.24	31.1
Sept. 23	27.9	0.70	4.05	0.51	0.15	23.9
Oct. 7	27.5	0.70	4.20	0.47	0.14	22.9
Semillon						
Aug. 18	21.4	0.65	3.45	0.49	0.22	31.0
Aug. 27	23.2	0.71	3.55	0.58	0.15	20.6
Sept. 12	26.5	0.60	3.60	0.56	0.14	20.0
Sept. 26	29.5	0.63	3.89	0.48	0.13	21.3
Oct. 14	27.9	0.56	4.00	0.48	0.11	18.6
Thompson Seedless						
Aug. 11	17.4	0.85	3.13	0.67	0.28	29.5
Aug. 25	19.7	0.77	3.20	0.64	0.24	27.3
Sept. 8	21.5	0.66	3.20	0.70	0.25	26.3
Sept. 22	22.8	0.65	3.40	0.57	0.17	23.0
Oct. 6	22.5	0.41	3.41	0.45	0.06	11.7
White Riesling						
Aug. 15	19.6	1.12	3.14	0.87	0.36	29.2
Aug. 28	21.2	0.97	3.40	0.79	0.26	24.8
Sept. 11	23.6	0.71	3.40	0.63	0.16	20.2
Sept. 25	23.1	0.63	3.50	0.56	0.09	13.8
Oct. 9	22.8	0.54	3.60	0.46	0.16?	25.8?

explained by differences in total acidity. The maturation studies and those conducted on ripe grapes were made in an attempt to elucidate these fluctuations. A summary of the more important European results is given in Table 4. These data show clearly that the amount of malic acid present differs greatly among the wine grape varieties, ranging from 12.8 to 46.7 percent for the sum of the tartrates and malates as malic acid. In Peynaud's data the percent is fairly constant for the same variety in different years. In his work the Merlot and Semillon varieties appear to be low in malate while Malbec, Petite Verdot, Verdot Colon, St. Macaire and Cabernet Sauvignon are high. Cabernet franc and Sauvignon blanc occupy intermediate positions.

The differences between varieties develop early in the season. The results of five years' work with Thompson Seedless grapes (Table 5) show that this variety has the highest tartrate content at maturity. Examination of the maturity data also show that it was the highest in tartrate in July, when the sugar content was below 10 percent. The mechanism favoring the accumulation of organic acids in the fruit obviously operates differently with this variety than with others, or the enzyme system favoring disappearance of organic acids from the fruit favors malic acid in this case and not in others.

A summary of the available information on the malic acid content of grapes grown at Davis is given in Table

TABLE 4

Malic Acid Content of Some European Grapes at Maturity

Variety and Source	Year	Tartrates as tartaric	Malates as malic	Malates as percent of total	pH
		percent	*percent*		
Negrara, Mach *(7)*	1868	0.42	0.38	46.7	.....
Malbec, Peynaud * *(9)*	1937	0.69	0.37	34.2	3.45
	1938	0.68	0.33	32.4	3.33
Merlot, Peynaud * *(9)*	1937	0.64	0.13	16.5	3.53
	1938	0.93	0.16	14.4	3.34
Cabernet franc, Peynaud * *(9)*	1937	0.56	0.26	32.5	3.38
	1938	0.97	0.26	20.8	3.36
Sauvignon blanc, Peynaud * *(9)*	1937	0.67	0.18	20.4	3.54
	1938	0.64	0.30	31.3	3.40
Semillon, Peynaud * *(9)*	1937	0.63	0.10	12.8	3.45
	1938	0.69	0.13	15.5	3.22
Petite Verdot, Peynaud * *(9)*	1938	0.86	0.47	34.5	3.27
Verdot Colon, Peynaud * *(9)*	1938	0.77	0.50	38.7	3.22
St. Macaire, Peynaud * *(9)*	1938	0.90	0.51	35.6	3.11
Cabernet Sauv., Peynaud * *(9)*	1938	0.85	0.60	40.6	3.42

* In Peynaud's data total acidity includes citric acid.

TABLE 5

Malic and Tartaric Acid Contents of Grapes Grown at Davis, California

Variety	Year	Balling, degree	pH	Tartrates as tartaric, percent	Malates as malic, percent	Malates as percent of total	Titratable acidity due to tartrates, percent
Barbera	1941	20-25	3.02	1.07	0.57	24.8	58.8
	1948	19.0	3.10	0.90	0.35	28.0	65.6
Cabernet Sauvignon	1948	22.2	3.80	0.59	0.28	32.2	56.8
Carignane	1939	20-25	3.53	0.41	0.32	43.8	48.3
	1940	20-25	3.47	0.71	0.43	37.7	53.1
	1941	20-25	3.26	0.61	0.47	43.5	48.3
	1947	21.4	3.51	0.56	0.15	21.1?	?
	1948	19.4	3.69	0.45	0.26	36.6	53.5
Chasselas doré	1939	20-25	3.54	0.52	0.19	26.8	65.4
Flame Tokay	1939	20-25	3.58	0.43	0.32	42.6	56.1
	1940	20-25	3.79	0.49	0.26	34.6	55.2
Mission	1940	20-25	3.60	0.49	0.32	39.5	50.1
	1941	20-25	3.36	0.61	0.47	43.5	48.9
	1948	19.7	3.71	0.44	0.22	33.3	57.2
Muscat of Alexandria	1940	20-25	3.57	0.57	0.29	33.7	57.5
	1941	20-25	3.36	0.49	0.17	25.8	67.3
	1948	20.4	3.58	0.67	0.26	27.0	64.9
Sauvignon vert	1948	17.8	4.03	0.60	0.45	42.8	44.3
Semillon	1947 [f]	21.4	3.45	0.47	0.22	31.9	59.6
	1947 [g]	23.2	3.55	0.58	0.15	20.5	71.4?
	1948 [h]	20.9	3.81	0.63	0.26	29.2	60.5
Sylvaner	1940	20-25	3.60	0.62	0.45	42.1	48.7
	1941	20-25	3.32	0.68	0.55	44.6	49.8
	1948	21.0	3.70	0.55	0.16	22.5	67.7
Thompson Seedless	1939	20-25	3.27	0.64	0.15	19.0	77.1
	1940	20-25	3.38	0.72	0.18	20.0	74.3
	1941	20-25	3.13	0.77	0.23	23.0	71.4
	1947	23.0	3.40	0.55	0.14	20.3	74.8
	1948	22.5	3.70	0.73	0.12	14.1	80.9
Valdepenas	1940	20-25	3.67	0.59	0.35	37.2	53.2
	1941	20-25	3.34	0.59	0.36	37.9	54.1
	1948	18.9	3.53	0.56	0.37	39.8	51.2

[f] Harvested Aug. 18. [g] Harvested Aug. 27. [h] Harvested Oct. 13.

5. Thompson Seedless has, as indicated above, the lowest malic acid content and the highest percentage of the titratable acidity resulting from tartrates. Other varieties of low malic acid content include Barbera, Chasselas dore, Muscat of Alexandria, and Semillon. High malic acid varieties are Carignane, Flame Tokay, Mission, Sauvignon vert, and Sylvaner. Cabernet Sauvignon and Valdepenas occupy an intermediate position.

Génevois *(4)* has reviewed the literature on the respiration of malic acid during the ripening of grapes. On purely physiological grounds, grapes would be expected to retain more malic acid in cool years than in warm years. However, the actual data of Peynaud *(9)* do not show uniform relation between the amount retained in the cool 1938 season and the warm year of 1937. The data presented in Table 5 have also been inspected to determine whether there is any uniform difference in the percent malates or in the percent titratable acidity due to malates. None has been found.

The failure to obtain the expected low malate content in warm years may be due to the very much earlier harvesting in such seasons. Preliminary studies during 1949 also failed to show the expected higher malic acid content in grapes of the same variety, grown in a cool region as compared to a warm one. Again, however, the difficulty may lie in the earlier harvesting of the warm-region grapes.

Both Génevois *(5)* and Peynaud *(9)* believe that the mixed plantings at Bordeaux are due to the desire of the growers to provide musts that will produce wines of a reasonably low total acidity every year. The climate at Bordeaux is quite cool (in comparison with most of the viticultural regions of California, for example) and there is frequently too high a titratable acidity in the musts and in their young wines. If the grape crush in the cool years includes some varieties with a high malic acid content, the acidity will be reduced in the finished wine as a result of the fermentation of the excess malic acid by various micro-organisms. In a warm year, the low malic acid varieties will help to maintain the total acidity (since tartaric acid is not respired in grapes until a very high temperature is reached). Moreover, the warm weather will induce the respiration of much of the malic acid from the high malic acid grapes prior to harvesting. It is probable, therefore, that high tartaric acid varieties should be planted in California. The climatic conditions of California are much warmer than those of European countries. For wine production, our grapes generally have too little acidity. Varieties with a high tartaric acid are much more likely to retain their acidity not only during ripening but also in the wine, all other factors being equal. Furthermore, malic acid is a weaker acid than tartaric (pK_{a_1} 3.41 versus 2.98) and, with the same buffer system, varieties with a high percentage of tartaric acid would be expected to have a lower pH than those with a low percentage.

CHANGES DURING FERMENTATION AND AGING

Peynaud *(9)* found losses in malic acid during fermentation of from 10.8 to 14.5 percent with five Bordeaux varieties but did not correlate them with the initial malic acid content. Using fifteen different yeasts, he found a loss during fermentation of 14.5 to 23.6 percent.

Malic acid is subject to bacterial attack. Normally this occurs after the primary fermentation during aging of the wines. However, at Davis, the author *(1)* found very high lactic acid content shortly after the finish of alcoholic fermentation. This is believed to be the result of bacterial action on malic acid during the primary fermentation. The importance and danger of the malo-lactic fermentation in California wines has been discussed in the same article. Again a high tartaric acid variety seems to be preferable in California, as this acid is much less subject to bacterial attack.

Potassium acid tartrate is more soluble in the presence of malic acid. A large malo-lactic fermentation will thus lead to the precipitation of potassium acid tartrate and a further reduction in acidity. While this would be desirable in very cool seasons or regions, it is not often favorable to the quality of California wines.

SUMMARY

Peynaud's procedure for the determination of malic acid has given satisfactory results only by removing the tartrates prior to the oxidation.

Malic acid is present in amounts of up to 50 percent of the total titratable acidity when grapes start to ripen. During ripening malic acid decreases (1) on a per berry basis, (2) as percent per ml. of juice, and (3) as percent of the sum of tartrates and malates. At maturity from ten to forty percent of the acidity is due to malic acid. A marked difference exists in the quantity of malic acid present in the several varieties of grapes. This difference appears early in the season and is maintained throughout ripening.

Although warm seasons and regions would be expected to produce grapes with a lower percentage of malic acid, the results obtained here, as well as those reported in the literature, do not clearly establish such a difference. However, in California, where warm climatic conditions prevail, it would be preferable to grow high tartaric acid varieties, other factors (pH, flavor, color, etc.) being equal.

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Measuring the Firmness of Cooked Apple Tissues

R. T. WHITTENBERGER

Eastern Regional Research Laboratory,[a] Philadelphia, Pennsylvania

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Cylindrical test specimens prepared from raw apples, are evacuated, cooked, and then compressed with a Delaware jelly-strength tester. The pressure-compression curves obtained show both quantitative and qualitative differences in firmness. The compression and tensile strength of cooked tissues are inversely proportional. Certain factors which affect firmness are discussed. With slight modification, the test is applicable to the measurement of firmness of potato, carrot, cucumber and other fleshy plant tissues.

Preliminary studies on the altering of apple tissue texture by treatment with calcium salts were handicapped by the lack of a suitable instrumental method for measuring tissue firmness. Most of the previously described methods *(2)* were applicable only to raw tissues, and the use of a penetrometer on calcium treated cooked slices did not always give a true measure of firmness, especially if the slices were surface-toughened.

The method described below was designed to measure the firmness of cooked tissues. In brief, the method consists in cutting test specimens from raw tissues, and in treating, cooking, and finally compressing the specimens. With the method it is possible to detect slight changes in firmness associated with different experimental treatments.

APPARATUS

The Delaware jelly-strength tester *(1, 6)*, which is essentially a glass syringe (inverted and clamped) to which air pressure is applied at the tip for forcing out the plunger, is used to compress a cylindrical specimen (Figure 1). Air pressure is reduced to approximately 25 pounds per square inch by a reducing valve, after which its flow is regulated with a needle valve. The flow is adjusted so that the pressure on the plunger increases almost linearly with time, as indicated by a column of carbon tetrachloride (CCl_4). About 30 seconds should be required to attain a pressure of 50 cm. of CCl_4. The plunger is marked in such a manner that its movement can be measured from readings on a millimeter scale fastened to the transparent barrel. The readings are facilitated through the use of a magnifying lens and a telecentric stop *(3)*. The glass head of the plunger is ground flat. Its diameter, 21 mm., is greater than that of the test specimen. The effective weight of the plunger which compresses the specimen before air pressure is applied, is equivalent to a pressure of approximately 5 cm. of CCl_4.

PROCEDURE

The procedure is divided into two phases, the preparation and pretreatment of specimens, and the measurement of compression. In order to obtain comparable results, it is necessary to pretreat (e.g. evacuate and cook) all specimens in the same manner. The pretreatments outlined below are not necessarily optimum for other studies, but in the present case they served well.

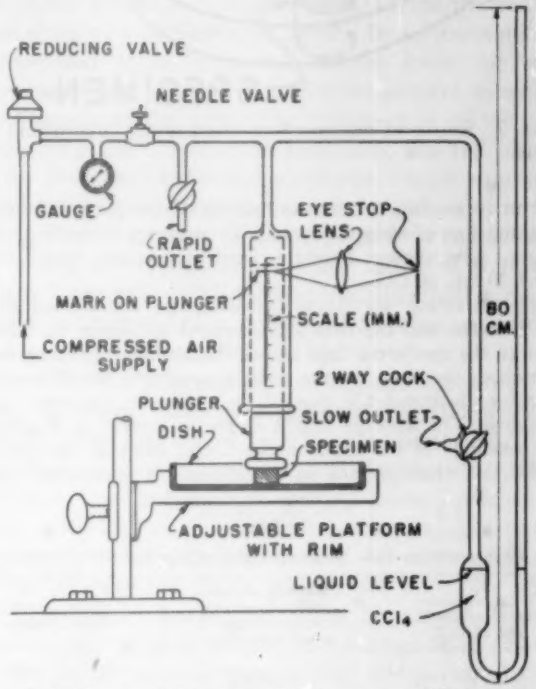

FIG. 1. Delaware jelly-strength tester as used for measuring tissue firmness. The various parts are not drawn to proportion.

To determine the firmness of cooked tissues from a fairly uniform lot of apples, seven raw apples are taken at random. If the lot is heterogeneous with respect to size and maturity, more than seven should be taken. Each apple is placed in turn with its stem-end up on a cutting block. With a long thin-bladed knife two parallel cuts about 2 cm. apart are made completely through the center of the apple from its stem end to its calyx end. The radial longitudinal slice thus formed should include both the stem and calyx. The slice is laid flat on the cutting block and from its cheek tissue, about 2 mm. inside the skin and midway between the stem and calyx, a cylinder 13.5 mm. in diameter is cut with a sharp cork borer (Figure 2). This cylinder is placed in a hole in a trimming block, which is a block of maple wood about 10 mm. thick containing a hole 13.5 mm. in diameter. Both protruding ends of the cylinder are cut off with a thin razor blade drawn flush along each surface of the block. The resulting right cylinder is approximately 10.7 mm. high. One cylinder from each of the seven apples is prepared in this manner.

The seven cylinders are placed in 100 ml. of distilled water and evacuated until they sink when the vacuum is broken. They are then boiled gently for exactly five minutes, after which they are cooled to room temperature.

A single cylinder is lifted carefully with a spatula to a petri dish fitted snugly within a recess in the platform (Figure 1) of the jelly-strength tester. The platform is adjustable either in a vertical or lateral direction. The petri dish has a flat, ground surface at its center to prevent slipping of the specimen. The specimen, resting on one of its ends, is centered beneath the manually lifted plunger, and the plunger is lowered slowly until it just touches the top of the specimen. The plunger height then is read from the millimeter scale to the nearest 0.1 mm. The plunger is released and is permitted to rest on the specimen

[a] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

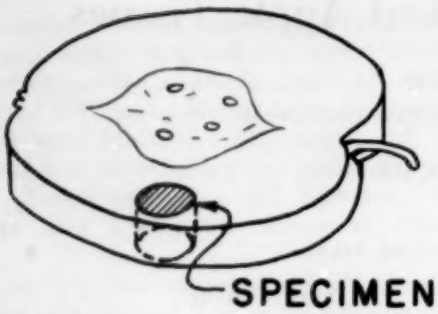

FIG. 2. Source of test specimen from radial-longitudinal slice of apple.

for 8 to 10 seconds. A second reading of the plunger height is made and immediately afterwards air pressure is applied to the plunger. The plunger height is read at pressure values of 10, 20 and 40 cm. of CCl_4.

The remaining six tissue cylinders are compressed in the same manner, and the data are averaged as shown in Table 1. If one of the specimens fails before the planned highest pressure is reached, the data on the softest specimen which remained intact is substituted for that of the collapsed specimen in the averaging. The average height of the specimens is determined from readings of the scale on the barrel made in the presence (initial test reading) and in the absence of specimens. Specimens may shrink or swell, depending upon their treatment. The average percent compression is then computed for the different pressures. The data are plotted as in Figure 3. For comparative purposes, the percent compression at a total pressure of 25 cm. of CCl_4 (equivalent to a force of about 43 grams or 0.3 g./mm^2) is used to indicate the firmness of the samples (Tables 2 and 3).

TABLE 1

Representative Data on Compression of Cooked Apple Tissue

Total pressure, on specimen,[b] cm. of CCl_4	Reading of mm. scale on syringe barrel, average of seven specimens	Average net compression	
		mm.	*percent*
0	1.6 [c]		
5	2.5	0.9	8
15	3.6	2.0	19
25	4.9	3.3	31
45	6.9	5.3	50

[b] The weight of the plunger resting on the specimen was equivalent to 5 cm. of CCl_4. This has been allowed for in the pressure values listed.

[c] The scale reading in the absence of specimens was 12.3 mm. Therefore, the average height of uncompressed specimens was 12.3 — 1.6 = 10.7 mm.

RESULTS AND DISCUSSION

Reliability of Test. Uniformity in experimental material may be approached by using tissues from the same apple for different treatments. For example, six or more tissue cylinders may be prepared from each of seven apples, one cylinder from each being used for a treatment. The evacuating and infiltrating of tissues facilitate the placement of reagents near their reaction sites. Solutions of reagents such as calcium chloride, ammonium oxalate, pectase, and sucrose may be distributed more uniformly through the tissues.

The reliability of the test is indicated by the statistically treated data of Table 3, columns 2 and 3. Since the differences in firmness between lots of apples were great (compression ranged from 6 to 40 percent in the eight lots shown in Table 3), the testing of as few as seven apples from each lot often was adequate for revealing statistically significant differences. Of course, if the differences in firmness are slight, or if greater accuracy is desired, more than seven apples should be tested.

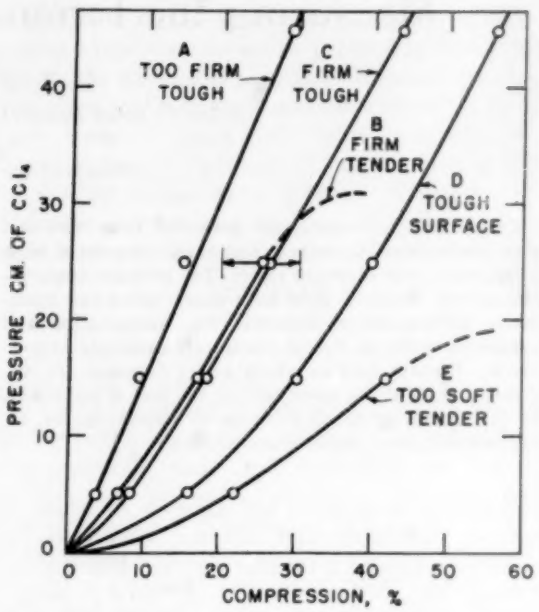

FIG. 3. Representative data showing compression of cooked apple tissues at various pressures. The curves illustrate qualitative as well as quantitative differences in firmness. Zone of optimum firmness at 25 cm. pressure is indicated by arrows through curves B and C.

Optimum Firmness. Since personal opinions are involved in estimating the most desirable degree of firmness, complete agreement on the concept is not to be expected. It is said that the consuming public objects more strongly to tissues which are too firm or tough than to those which are too soft. Furthermore, the concept of optimum firmness may vary with the maturity and variety of apple, or the concept may be influenced by differences in other characteristics of the tissues.

For the objective characterization of optimum firmness, samples were tested by the present method, by the

TABLE 2

Comparison of Objective (Compression) and Subjective Methods for Evaluating the Firmness of Cooked Apple Tissues

Sample	Method	
	Objective [d]	Subjective [e]
	Compression, percent at 25 cm. pressure	Firmness score
1	60	1
2	59	1
3	33	2
4	33	2+
5	31	2
6	27	3
7	24	3
8	22	3
9	21	4
10	17	5
11	12	4
12	11	5
13	7	5

[d] Test specimens, prepared from the raw tissues, were cooked in a boiling water bath for 20 minutes with the slices used for subjective scoring.

[e] Firmness was rated on a scale of 0 to 5, where 0 = mushy or sauce consistency, 3 = optimum firmness, and 5 = tough.

TABLE 3

Data Illustrating Relationships Among the Compression, Tensile, and Magness-Taylor Tests as Applied to Apple Tissues [f]

Apple Samples [g]	Cooked Tissues [h]			Raw Tissues
	Compression		Tensile strength, g./mm.[2]	Magness-Taylor test, pounds
	Percent at pressure of 25 cm.	Standard error		
Stayman Winesap	40	2.6	0.27	11.1
Red Delicious A	39	2.3	0.32	9.8
Jonathan A	34	2.8	0.57	10.0
Jonathan B	30	1.6	0.63	10.4
McIntosh	30	3.9	0.45	9.3
Stark	28	3.0	0.39	12.7
Red Delicious B	11	1.6	2.08	13.1
York Imperial	6	0.6	2.01	22.0

[f] The coefficient of correlation between the compression and tensile strength of cooked tissues was —0.94. The correlation coefficient between the compression of cooked tissues and the Magness-Taylor test on raw tissues was —0.81.

[g] The data do not indicate necessarily the comparative firmness of the apple varieties, but apply only to these particular samples following storage for about five months at 34° F. The York Imperial and McIntosh apples were picked at a less mature stage than were the other varieties.

[h] For the compression test of each variety, one specimen from each of seven apples was used. For the tensile test, two specimens from each of seven apples were used.

subjective method of Hills et al. *(4)*, and in some cases by a commercial pie baker. In general, tissues of optimum firmness (subjective rating) were compressed approximately 20 to 30 percent by a pressure of 25 cm. of CCl_4 (Table *2*). Good agreement was obtained between the results of the instrumental and subjective methods, although the difficulty of subjectively estimating differences in firmness of comparatively firm tissues was apparent.

Qualitative Differences in Firmness. In addition to differing quantitatively in firmness, tissues may also show a qualitative difference. Equally firm tissues, as evidenced by their percent compression, may be either tough or tender. These qualitative differences are revealed in at least some cases by the present method of testing.

Tissues which are tough remain completely coherent, even though they may be compressed extensively by comparatively high pressure. After a recovery period the same tissues may be recompressed to give approximately the same result as originally. On the other hand, tender tissues collapse completely and irreversibly at only moderate pressure. In subjective terms such tissues "melt in the mouth" as the cells separate from each other and the tissues lose their continuity.

Qualitative differences in firmness are illustrated by the pressure-compression curves of Figure 3. Curves B and E show the compression of tender tissues when subjected to increasing pressure, and curves A, C and D illustrate the compression of comparatively tough tissues. The collapse of the tissues which gave curves B and E is indicated by the dashed portion of the curve. Although the tissues giving curves B and C were compressed to approximately the same extent by a pressure of 25 cm., the tissues of B collapsed when subjected to a greater pressure whereas those of C remained coherent.

A comparison of curves C and D illustrates an additional qualitative difference in firmness. The tissues of C were uniformly firm throughout their volume. In contrast, the tissues of D were surface-toughened but soft internally. In this case a slight pressure produced a comparatively large amount of compression.

Factors Affecting Firmness. Tissue firmness was affected by a number of factors which may vary either with the experimental material or with the conditions of the test. In general, there was no consistent difference in the firmness of cooked tissues from the red and green sides of a symmetrical apple. In an asymmetrical or lop-sided apple, however, tissues from the small side were on the average from 5 to 10 percent more firm than those from the large side, irrespective of the color of the two sides. Usually the small side was red. Variation in firmness of the tissue cylinders from one apple was about half as great as that of the cylinders from a group of seven apples. Cylinders cut in a radial direction (cork borer pushed from the cheek skin toward the core) were about 15 percent less firm than cylinders cut and compressed in a tangential direction, as is done in the present method.

The effect on firmness of soaking or evacuating raw tissues in water varied with the variety and maturity of apple. The soaking of fully mature tissues for three hours in some cases resulted in a 50 percent decrease in firmness. The firmness of cooked tissues varied inversely with the temperature of the tissues, cool tissues being more firm than warm tissues. In one case lowering the temperature from 60° to 25° C. (140° F. to 77° F.) increased firmness by about 20 percent. Finally, the rate of applying pressure to the tissues affected their firmness values, a slow rate giving comparatively low values. For example, with comparatively soft tissues, compression was increased about 25 percent by doubling the time required to attain a pressure of 50 cm. of CCl_4.

Correlation With Other Tests. The results obtained by three different test methods on the same apple tissues are shown in Table 3. The coefficient of correlation between the Magness-Taylor *(5)* test (firmness of raw tissues) and the compression test (firmness of tissues cooked in distilled water) was —0.81 for the samples of Table 3. These samples had been in cold storage for about five months and for the most part were of a low degree of firmness. However, in other samples, especially in freshly harvested summer or early fall varieties such as Yellow Transparent, Gravenstein, Wealthy, Williams Early Red, and McIntosh, firm raw tissues disintegrated completely when cooked in distilled water, and there was no correlation between the Magness-Taylor and compression tests. Yet if the same tissues were cooked in 0.1 percent calcium chloride solution (whose effect is similar to that of the tap water used in some processing plants), no disintegration occurred and the correlation between the two tests was good. Detailed data on the firmness of raw and cooked tissues as affected by apple variety, storage conditions, and calcium ion and other treatments, will be reported in a subsequent paper.

The compressibility and the tensile strength of the cooked tissues shown in Table 3 were inversely related, the coefficient of correlation being —0.94. The method of Personius and Sharp *(7)* was used for determining the tensile strength. Microscopic examination of the

separated surfaces of tissue dumbbells disclosed no broken cell walls. The break in the specimen was accomplished through the separation of whole, intact cells. Similar observations were recorded for tissues which were compressed beyond their breaking point. Both the tensile strength (cells pulled apart) and the compression (cells pushed together) measurements were, therefore, measurements of the strength of the middle lamella. These observations thus emphasized the importance of the intercellular cement as a determiner of tissue texture.

Testing Other Plant Tissues. The present method may be used without modification to determine the comparative firmness of other fleshy plant tissues. In most cases, however, it was advantageous to adjust the test conditions to accommodate different ranges in firmness exhibited by different tissues. This was done principally by varying the pressure, the cooking period, and the diameter of the test specimen. For tissues which usually were firm or hard, such as white potatoes, carrots, and beets, tissue cylinders 8.7 mm. in diameter and 10.7 mm. high were cut and boiled for 30 minutes. The percent compression at a pressure of 55 cm. of CCl_4 was measured. For tissues which were somewhat softer, such as cucumber, cylinders 10.0 mm. in diameter and 10.7 mm. high were prepared and tested under the same conditions as were the potatoes.

The test conditions outlined above for potatoes, carrots, beets, and cucumbers were not necessarily optimum, for only a limited number of tests were carried out. Yet the results indicated the feasibility of the test and suggested its possible application in determining such qualities as the mealiness of potato tissue.

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A Study of the Stabilization of Fats Incorporated in Candy[a]

HELEN M. ROBINSON

Agricultural Chemical Research Division,[c] Southern Regional Research Laboratory, New Orleans, Louisiana

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Oxidation of unsaturated fats, formation of free fatty acids and the phenomenon characterized as "reversion" may all produce undesirable flavor and odor when used in candies. The usefulness of permissible antioxidants in preventing the first two types of changes has been investigated. Copper stearate has been added to the fats to accelerate the deteriorative reactions.

Candies containing fats are subject to deterioration, generally referred to as rancidity spoilage. The term rancidity has been used by the industry to mean deterioration in odor or flavor without reference to the particular kind of rancidity or its cause.

The objectionable flavors and odors observed may be due to one or more of several different chemical changes in the fat, designated as: a) oxidative, b) hydrolytic, c) ketonic, d) reversion. Oxidative and hydrolytic rancidity are the most common types in fat-containing products in general.

Candy manufacturers seldom care which type of rancidity is involved when their candy has an off-odor or taste. The product is unsalable and they want to know what to do about protecting it from spoilage. However, since each type of rancidity will require a different remedy, it is necessary to determine chemically which of the types of rancidity is concerned.

An accelerated aging method involving the use of added copper stearate to hardened vegetable oils and to butter has been used, and the fats thus treated have been incorporated in candy. Various antioxidants have been used in the treated candies to attempt to prevent deterioration both in candies containing the accelerator and those without.

Because oxidative and hydrolytic rancidity are more generally encountered and of greater importance to the candy industry the present studies have been limited to these two types.

Oxidative Rancidity, the type most generally encountered, occurs spontaneously when the unsaturated fat is exposed to air. The rate at which it develops will vary with the conditions of the exposure. Fats are complex mixtures of glycerides which are built up from glycerol in combination with saturated and unsaturated fatty acids. The saturated acids which are relatively stable when exposed alone to air may be oxidized in the presence of oxidation products of the unsaturated acids. The predominance of aldehydes is characteristic of oxidative rancidity. The rancid odor and flavor of

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[c] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

fats undergoing this form of decomposition has been reported by Powick *(3)* to be similar to that of heptyl and nonyl aldehydes.

Hydrolytic Rancidity is met with more often in butter fat than in the hardened vegetable oils *(6)*. Hydrolysis of butter is promoted by water or the presence of lipases. These enzymes are natural constituents carried over from the milk or may be of bacterial origin. If by hydrolysis fatty acids with less than 12 carbon atoms are liberated, this spoilage becomes perceptible organoleptically. This is especially the case for butyric acid *(3)*. High temperatures will reduce the activity of lipase [4] but when butter is used in candies it is usually added after most of the cooking has been completed. Butter creams are often made by adding the butter after the cook has cooled to 110° F. (43° C.) and has been removed from the fire. Caramels may have butter added at about the same temperature, and then be cooked for less than a minute. These conditions do not ensure inactivation of the lipase.

Ketonic Rancidity is characterized by a distinctive but not always unpleasant ester-like odor, accompanied by a pungent, unpleasant taste. This type of rancidity is produced by a particular group of micro-organisms. Decomposition of the fat by beta-oxidation of the ammonium or sodium salts of saturated fatty acids gives small yields of methyl ketones. Ketonic rancidity is not common, as ketones are not produced by the action of micro-organisms on acids of higher molecular weight than myristic acid. Fats which do not contain the lower fatty acids do not develop ketonic rancidity. Under the sanitary conditions which should be maintained in the storage of candy, conditions for ketonic rancidity would not exist.

Reversion is a term usually used in reference to the rancidity of soybean oil and fish oils. A great deal of work has been done by workers in this field. Spannuth *(6)* says "the term 'reversion' is a misnomer, because the objectionable flavor and odor is not characteristically normal to the fresh fat. The evidence is growing, as well as the list of converts, that the phenomenon is one of extremely limited oxidation involving linolenic acid. Trace ingredients may or may not be involved." Soya oil is used only to a limited extent in the candy industry. There are no methods other than organoleptic taste testing for measurement of this particular type of rancidity.

EXPERIMENTAL METHODS

Development of rancidity in fats in candies stored at ordinary temperatures is slow, requiring months to complete, so it is desirable to obtain preliminary information based on an accelerated method. Neither the active oxygen method nor the Schaal oven test are suitable for use on candy. At the temperatures used in either of these methods the candy being tested would break down physically, the fat would separate, and the conditions would not correspond to those under which deterioration of the stored candies actually occurs, even under extreme storage conditions.

A number of chemical pro-oxidants are known which can be added to the fat to accelerate the reaction. Once deterioration starts and peroxides are formed, these oxidation products themselves act as powerful pro-oxidants. Ozone speeds up the reaction, as do also ascorbic acid and cysteine in fats emulsified in water *(5)*. The only suitable pro-oxidants for use in fats to be incorporated in candies were the metals. Work has been done along this line using strips of metals immersed in the fatty material. In the case of milk fat, a copper sulfate solution was added to the milk *(7, 8)*.

Traces of copper were used for the accelerated tests in the present work, being added to the melted fat in the form of copper stearate. After preliminary work with various amounts, 10 p.p.m. of copper (based on the weight of the fat) was selected as the most suitable amount to use. The samples of candies were stored in air ovens at 86° F. (30° C.) and determinations were made periodically of peroxide values by the method of Wheeler *(10)* and of free fatty acids *(2)*. Fat samples both with and without antioxidants were tested in the same way.

In analyzing the candies the fat was extracted by dissolving 50-100 g. of the candy in hot water [up to 158° F. (70° C.)]. This extract was allowed to stand in the refrigerator for 2-3 hours until most of the fat formed a crust on the surface and could be skimmed off and dissolved in chloroform. The water solution of candy was extracted with an equal or larger volume of chloroform and combined with the chloroform solution of the fat. This extraction requires some care to avoid the formation of emulsions.

The combined chloroform extract finally obtained was divided into two portions. The first was evaporated on the steam bath to remove the solvent, weighed to determine the amount of fat recovered, and titrated for free fatty acids. The second portion was evaporated by passing in a current of nitrogen until the volume is reduced to approximately 20 ml. Thirty ml. of acetic acid was then added, and the peroxides were determined.

To test the reliability of the extraction procedure, oil having a determined peroxide value of 3.8 was incorporated in a cream fondant by regular candy making procedures and the resulting candies were extracted immediately after being prepared. Seventy-five to 90 percent of the oil was recovered and found to have a peroxide value of 4.1.

The peroxide test of Wheeler *(10)* has been used as an indication of oxidative rancidity and the A.O.C.S. official method for free fatty acid determination to check on the hydrolytic rancidity.

EXPERIMENTAL RESULTS

Hardened Vegetable Oils. Traces of copper were added to an excellent grade of a hardened vegetable oil widely used in the candy industry and samples were stored at 86° F. (30° C.). The rate of deterioration of the fat both with and without antioxidants was measured by both the peroxide method and free fatty acid determinations. This particular fat was very slow to deteriorate in the ovens, and its extreme stability was also shown by results of the active oxygen (Swift) test at 212° F. (100° C.). Plain hardened vegetable oil broke down (showed a peroxide value of 10) after oxygen was bubbled through it at 208° F. (97.7° C.) for 400 hours. When copper was added to this fat it broke down at 68 hours. Hardened vegetable oil with 0.10 percent lecithin did not reach the breakdown point either with or without copper after 600 hours. The presence of copper accelerates the breakdown in the plain fat but fat containing lecithin is resistant to the accelerating effect of copper. This is to be expected since lecithin is a good metal scavenger. It is usually

[4] A personal communication from Dr. George Greenbank, Division of Dairy Products Research Laboratories, Bureau of Dairy Industry, USDA, states the following conditions for the inactivation of milk lipase: Heating for 7.5-10 min. at 165° F. (73.9° C.) will destroy 50 percent activity, heating for the same period to 185° F. (85.0° C.) and to 200° F. (93.3° C.) will destroy 85-87 percent and practically all lipase activity, respectively.

added by the manufacturer to hardened vegetable oils of this type.

Series were run in the ovens at 30° C. on this commercial grade of hardened vegetable oil and for the same material furnished by the manufacturer without added lecithin. The material without lecithin was a very fresh sample sent to us immediately after manufacture. Up to 25 weeks the treated and untreated samples showed zero peroxide values. Another sample from the same manufacturer, but with the lecithin incorporated in it, had been in the laboratory about six months, stored in a refrigerator. This fat also showed a zero peroxide value, but up to the fourteenth week the free fatty acid values were from 0.40 to 0.70; and by the seventeenth week values ranged from 0.40 to 1.02. This fat had a slight rancid odor from about the tenth week and the appearance of many of the samples was flaky and yellowed.

Creamery Butter. Samples of fresh creamery butter containing amounts of antioxidants recommended by their manufacturers were stored in air ovens at approximately 86° F. (30° C.) and at intervals tested for free fatty acids and peroxide content. The results obtained are shown in Table 1. The butter was used without a copper accelerator, showed a definite peroxide test by the eighth week, but deterioration was slow with a value of 1.1 after 28 weeks. It had a definitely spoiled odor by the fifth or sixth week and at that time the free fatty acid values were high (see Table 2). The butter samples containing antioxidants showed no peroxide formation at all up to the twenty-eighth week with free fatty acid formation in the nineteenth week.

The addition of 4 p.p.m. of copper to the butter did not accelerate the reaction sufficiently. As shown in Table 1, the addition of 10 p.p.m. of copper produced considerable acceleration of deteriorative reactions. In Figure 1 the trends of these changes are indicated.

Antioxidative deterioration of fat usually shows an induction period. In this stage the oxidation is latent, the peroxide value low and the organoleptic properties either unchanged or very slightly changed. After this period the peroxide value usually increases sharply and spoilage is quite evident organoleptically.

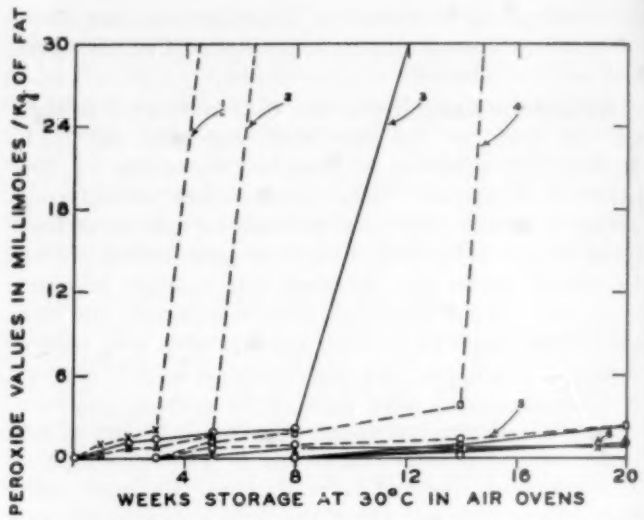

FIG. 1. Effects of various additions on the peroxide value of stored butter. 1. Butter plus 10 p.p.m. Cu. 2. Butter plus 10 p.p.m. Cu plus antioxidant mixture (see Table 2). 3. Butter plus 4 p.p.m. Cu. 4. Butter plus 10 p.p.m. Cu plus special oat flour. 5. Butter plus 10 p.p.m. Cu plus nordihydroguaiaretic acid. 6. Butter alone. Other solid lines which do not reach value greater than 2 in 20 weeks are butter samples with 4 p.p.m. copper and the same antioxidants listed above.

Since this work was to evaluate spoilage of fat in candy, a higher organoleptic point was taken than is usually mentioned for butter alone. Holm *(1)* states that butter is definitely tallowy at a peroxide value of about 2.5. In this work an arbitrary value of 20 was taken as at that value the off taste was definitely detectable in the finished candy.

From the data it can be seen that the butter sample with 10 p.p.m. copper started to deteriorate definitely by the first week and by the fifth week showed complete breakdown. Of the antioxidants used, the nordihydroguaiaretic acid and special oat flour were most effective in delaying the onset of oxidation. With these antioxidants the reaction did not begin until the fifth week, after which it was rapid with the oat flour, but very slow with nordihydroguaiaretic acid. The organoleptic endpoint of a peroxide value of 20 was not reached after 28 weeks. As seen in Figure 1 the butter containing oat flour had deteriorated completely in 14 to 22 weeks. The special antioxidant mixture used showed break-

TABLE 1

Effects of Various Antioxidants on the Peroxide Values of Butter After Storage

	Peroxide values [a]								
	Length of storage, in weeks								
	1	2	3	5	8	14	19	22	28
Butter alone	....	....	....	0.2	0.8	....	0.9	....	1.1
Butter plus 0.01 percent nordihydroguaiaretic acid	....	....	....	0	0	....	0	....	0
Butter plus 0.15 percent special oat flour	....	....	....	0	0	....	0	....	1.8
Butter plus 0.013 percent antioxidant mixture [b]	....	....	....	0	0	....	0	....	0
Butter plus 4 p.p.m. Cu	0	1.2	1.5	1.9	2.1	42.9	....	....	....
Butter plus 0.01 percent nordihydroguaiaretic acid	0	0	0	0	0	0.5	....	1.2	....
Butter plus 0.15 percent special oat flour	0	0	0	0	0	1.0	....	2.4	....
Butter plus 0.013 percent antioxidant mixture [b]	0	0	0	0	0	0.7	....	0.9	....
Butter plus 10 p.p.m. Cu	1.1	1.6	2.1	79.2	118.6	138.1	....	....	....
Butter plus 0.01 percent nordihydroguaiaretic acid	0	0	0	0.8	0.9	1.4	....	2.5	....
Butter plus 0.15 percent special oat flour	0	0	0	1.2	1.9	3.9	....	127	....
Butter plus 0.013 percent antioxidant mixture [b]	0.3	0.8	0.7	1.8	53.3	131.7	....	....	....

[a] Expressed in millimoles peroxide per kg. of fat. [b] Propyl gallate, cephalin, lecithin, alpha and gamma tocopherols and citric acid.

TABLE 2

Effects of Various Additions on the Free Fatty Acid Values of Stored Fats

	Peroxide values [a]									
	Length of storage, in weeks									
	Hardened Fat (no lecithin)		Vegetable oil (with lecithin)		Butter					
	16	25	14	17	4	6	11	15	19	28
Fat used, no addition	0.20	0.16	0.44	0.40	0.30	1.31	1.28	1.21	.....	.....
Fat plus 10 p.p.m. Cu	0.20	0.16	0.43	0.43	0.33	1.85	.....	3.32	.....	.....
Fat plus 0.01 percent nordihydroguaiaretic acid	.....	.....	.....	.....	.....	0	.....	0	0.61	10.36
Fat plus 10 p.p.m. Cu plus 0.01 percent nordihydroguaiaretic acid	0.16	0.18	0.69	0.60	.....	.....	.....	.....	.....	.....
Fat plus 0.15 percent oat flour	.....	.....	.....	.....	.....	0	.....	0	0.84	1.29
Fat plus 10 p.p.m. Cu plus 0.15 percent oat flour	0.22	0.17	0.43	0.44	.....	.....	.....	.....	.....	.....
Fat plus 0.013 per cent mixed antioxidant [b]	.....	.....	.....	.....	.....	0	.....	0	0.54	0.80

[a] Expressed as percent oleic acid. [b] Propyl gallate, lecithin, cephalin, alpha and gamma tocopherols and citric acid.

down from the start, becoming rancid between the fifth and eighth weeks.

Candies. A series of butter creams with added copper accelerator and antioxidants was put in storage. The tests run on the butter and butter with copper used in making the creams show similar results. The candy itself, without added copper, gave no peroxide value until the eighth week when the value was 1.0. A peroxide value of 20 considered to be the organoleptic end point was reached by the fifteenth week. This butter cream also showed definite hydrolytic rancidity by the sixth week, however. The butter creams with copper showed a shorter induction period, less than six weeks, and a much more rapid build-up of peroxide than the plain butter cream. The nordihydroguaiaretic acid and antioxidant mixture protected this cream against oxidative rancidity for the 15 weeks of the experiment, but the oat flour gave no protection.

The results in Figure 1 appeared to indicate that two of the antioxidants, nordihydroguaiaretic acid and special oat flour, gave protection against hydrolytic rancidity. However, later work did not bear this out.

An extensive series of caramel samples containing creamery butter has been prepared using eight antioxidants. So far there has been little indication of oxidative rancidity. At five weeks the butter used, tested alone, has a peroxide value of 0.7 and the sample with copper accelerator added has a value of 1.0 but none of the others, either the plain caramel or any of the samples with or without copper to which antioxidants have been added show any peroxide value. However, in the same period most of the samples show enough hydrolytic rancidity to be detected organoleptically.

It should be emphasized that all butter used was of 90 score and fresh, obtained from a large local dairy, and had an initial peroxide value of zero. Despite this fact the butter varied a little in length of time of induction period depending on the history of the butter itself and the cream from which it was made. The freshness of the butter is of prime importance.

DISCUSSION

The addition of 10 p.p.m. of copper to butter (Table 1) shortened the induction period from more than 28 to three weeks. The use of antioxidants in the presence of copper brought back the induction period in the case of nordihydroguaiaretic acid to almost its original length, over 28 weeks when copper had not been present; to 22 weeks in the case of oat flour, and to eight weeks in the case of the antioxidant mixture. Even in this last case the period was lengthened appreciably.

Butter used in making the butter creams had an induction period of approximately 22 weeks. The addition of 10 p.p.m. of copper shortened this to four weeks. The stored cream fondant made with this butter showed a longer period with zero peroxide value, but once oxidation began peroxide developed faster than in the butter tested alone. The induction period was 11 weeks. Fondant made with butter containing copper began to deteriorate sooner and the change was more rapid than in the unaccelerated test, as can be seen from the curves in Figure 2. Both nordihydroguaiaretic acid and the antioxidant mixture gave full protection for the length of time of the experiment. The special oat flour appears to have accelerated spoilage.

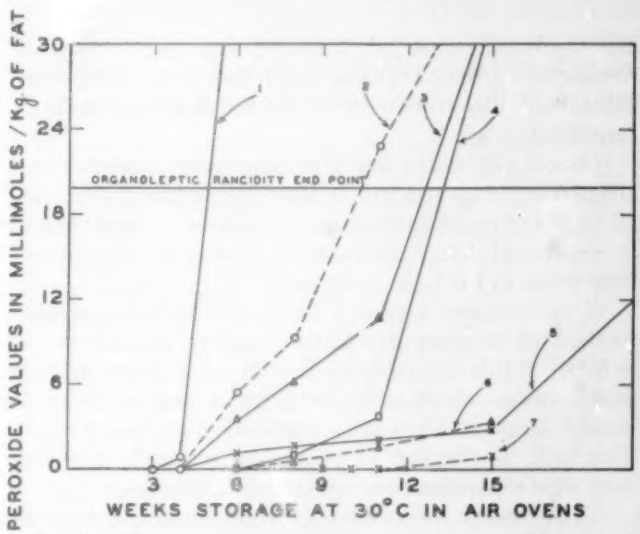

FIG. 2. Effects of various additions on the peroxide value of stored butter creams. 1. Butter plus 10 p.p.m. Cu. 2. Butter cream plus copper plus special oat flour. 3. Butter cream plus Cu. 4. Butter cream, no addition. 5. Butter, no addition. 6. Butter cream plus Cu plus antioxidant mixture (see Table 2). 7. Butter cream plus Cu plus nordihydroguaiaretic acid.

The series of butter caramels has not yet reached the point of providing significant data on oxidative ran-

cidity. In the preparation of these samples a batch of caramel was split in half. To one part was added butter plus antioxidant and to the other, butter plus antioxidant plus copper. These must be compared for each antioxidant. A similar series of butter creams is also underway.

It is evident from both the work on butter creams and on caramels that the use of antioxidants alone will not solve the problem. The lower fatty acids present in butter when freed by hydrolysis produce an unpleasant rancid odor and taste. As shown in Figure 3

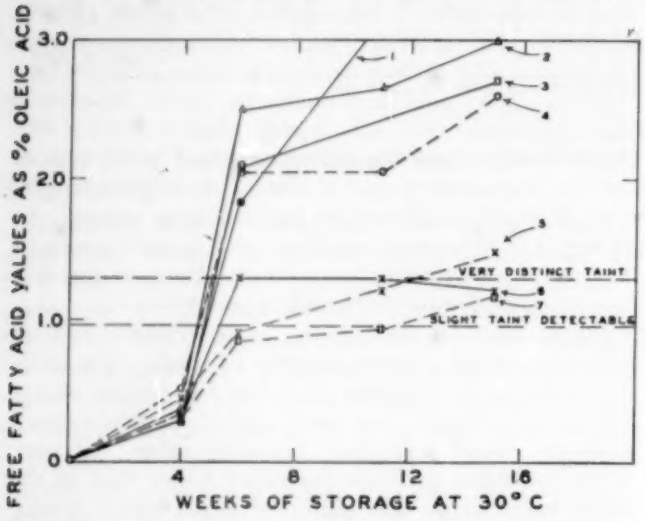

FIG. 3. Effects of various additions on the free fatty acid values of stored butter creams. 1. Butter plus 10 p.p.m. Cu. 2. Butter cream plus Cu. 3. Butter cream alone. 4. Butter cream plus Cu plus special oat flour. 5. Butter cream plus Cu plus nordihydroguaiaretic acid. 6. Butter alone. 7. Butter cream plus Cu plus antioxidant mixture (see Table 2).

and the work done in caramels, they are formed in caramels and butter creams much too soon. Still other substances may contribute to the burning after-taste of rancid fat.

Reinart *(4)* states that from the butter analyses and airbubbling experiments it may be deduced that stability of butter fat against auto-oxidation is variable and is dependent upon its content of highly unsaturated fatty acids in the C_{20}-C_{22} series.

Work is under way at present to adapt a suggested method of measuring volatile reducing substances. It is believed this can be used directly on a water solution of the candy which will save a great deal of time. It should measure all volatile reducing materials present. This will be checked of course by peroxide and free fatty acid determinations on the same candies.

All of the results obtained by accelerated test methods are of value primarily in exploring as rapidly as possible the nature of the deterioration of fats incorporated in candies, and possibilities of preventing it by antioxidants or other means. Final evolution of specific methods of protecting the fats can be established only by unaccelerated, long-time storage tests.

ACKNOWLEDGMENT

The author wishes to thank Mr. Frank G. Dollear for his advice and help in this work and Dr. W. G. Bickford and Miss Sara Fore of the Oil and Oilseed Division of the Southern Regional Research Laboratory for the work done with the Swift Stability Test (active oxygen method).

SUMMARY

1. A method of accelerating fat oxidation by the use of traces of copper, added as copper stearate, has been applied in studying the deterioration of fats in candies.

2. Using the accelerated method samples of butter and of hardened vegetable oils have been tested for peroxide values and free fatty acids after storage at 86° F. (30° C.) in air ovens.

3. The fats in butter creams and butter caramels were tested in the same way.

4. A series of antioxidants has been tested in the fats alone and in the fats with added copper, and with these same fats incorporated in candies. Hardened vegetable oils showed very little oxidative rancidity even with added copper so antioxidants were of little value. Butter with added copper shows definite peroxide values in a short period and this period can be materially lengthened by the use of antioxidants.

5. Final evaluation of the results must await the completion of the tests on candies without the added accelerator.

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Biological Factors in Damp Grain Deterioration[a,b]

MAX MILNER
Kansas Agricultural Experiment Station, Manhattan, Kansas

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A REVIEW

Moisture content is a major hazard in stored grain since it promotes deterioration due to biological processes. Respiratory activity and heating tendency increase sharply only when a critical moisture value is exceeded. The moisture content of stored grain is closely dependent on the interseed atmospheric humidity. Thus the germination of certain mold spores on and within the seed coats and the proliferation of mycelia commences at a rather well-defined humidity level which is in equilibrium with the various critical moisture contents characteristic of individual grains. The different kinds of microorganisms contaminating natural products including molds, yeasts and bacteria require different degrees of hydration for growth, and the hygroscopic moisture equilibrium of a material therefore will determine the type of spoilage which will occur. At elevated moisture values in initial storage stages, spontaneous heating of stored grain is due to the accumulating heat produced by growing molds.

Recognition of the role of fungi in the deteriorative process in damp grain has prompted investigations of the utility of chemical preservatives to control grain spoilage. The present status of these studies is reviewed.

The deleterious effect of moisture on stored grain probably has plagued man since he first domesticated cereal grains for food purposes. Only in the past several years, however, has a fairly clear picture emerged concerning the basic causes of grain deterioration due to excessive moisture content, under conditions where insect infestation is not involved.

An interesting anomaly has existed in this field during the past 30 years in that investigations have been carried out not only by botanists and plant physiologists, but also to a major extent by cereal chemists who have gone beyond their primary interest in the composition and technology of grains into studies of their respiratory and biological characteristics. In recent years a welcome ally has entered the field in the person of the plant mycologist, and the combined efforts of the two research interests have greatly clarified the problem of moisture deterioration in stored grain.

UTILITY OF RESPIRATION TECHNIQUES

The transfer of scientific inquiry in grain storage into the laboratory from the field and granary came about only at the turn of the century, when rapid progress was initiated toward our present understanding of this subject with the application of respiratory techniques to study the influence of moisture on grain metabolism. Thus a German worker, Kolkwitz, in 1901 *(14)* made the significant observation that dry barley seeds showed a considerable increase in respiratory activity if their moisture was raised from 15 to 16 percent. Kolkwitz found that grinding the grain further accelerated its respiration at the same moisture content, but that when toluene was applied to the actively respiring grain, a decrease in respiratory activity occurred. This worker attempted no explanation of these results, nor did subsequent workers in this field. A direct key to the modern concept of storage deterioration of grain thus lay neglected for many years.

Further elaboration of the relationship of moisture content to respiratory activity in grain was made in the now classical studies of Bailey *(4, 5, 6, 7, 8)* who initiated at the University of Minnesota a long-standing interest in this field which was carried on by a number of other workers *(9, 10, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27)*. His objective lay in establishing a rational basis for the moisture limits for various commercial grains whch are prescribed by the U. S. Grain Standards. For these investigations Bailey constructed respirometer vessels in which grain samples were maintained sealed for 48 hours or more at constant temperature, after which interval the carbon dioxide produced was swept out and determined by absorption in barium hydroxide. The extensive work of Bailey and his associates established clearly that if the moisture content of normally dry grain was elevated, the respiration increased only slowly until a certain critical value was reached, beyond which a rapid increase in carbon dioxide production occurred. The results obtained for a number of cereals as well as flaxseed are summarized in Figure 1. It is to be noted that most cereal grains show rapid respiratory increases in the critical moisture

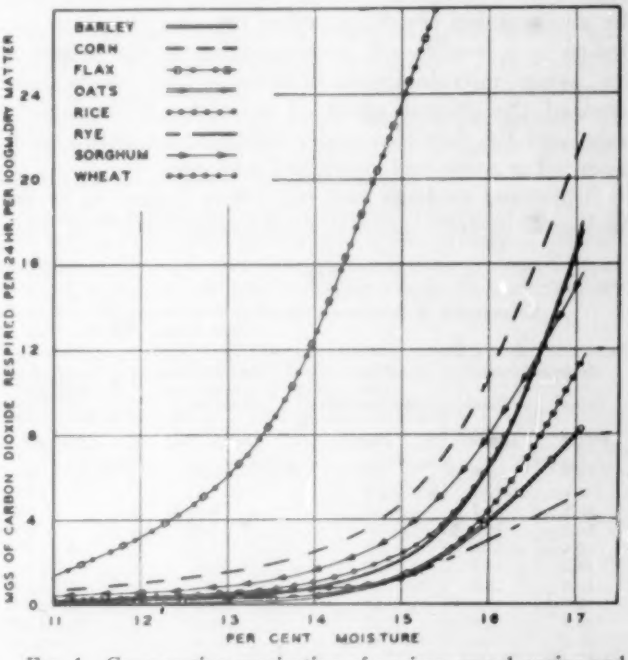

FIG. 1. Comparative respiration of various cereal grains and flaxseed. Data of Bailey *(6)*.

[a] Contribution No. 186, Department of Milling Industry.

[b] Presented before the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 22, 1950.

range of 14 to 15 percent, while flaxseed exhibited a respiratory increase below 11 percent moisture. To explain the respiratory increase at a critical moisture value Bailey advanced the hypothesis that a change in the bound- and free-water relationships occurs at this point. It was proposed that in this moisture region the discontinuous colloidal structure of the dry seed is transformed into a continuous gel through which metabolic interchange could readily occur, thus causing a sharp rise in the respiratory activity of the embryo.

CRITICAL MOISTURE AND THE ROLE OF FUNGI

The techniques developed by Bailey and coworkers were used by others to establish the respiratory characteristics of other crops, including sorghum *(11)* and soybeans *(27)*. Gilman and his group at Iowa State College, questioned the theory that respiratory increases may be attributed solely to seed metabolism *(12)* and suggested that the earlier workers had overlooked the fact that mold growth was initiated at moisture values where respiratory increases were observed. Later, questions were raised regarding the static method to determine respiration, for the reason that it did not take into account the possibility that respiratory activity at any moisture value might not be constant with time.

These criticisms led eventually to the adoption of new respiration techniques which provided continuous aeration of seed samples maintained at constant temperature with continuous determination of carbon dioxide production, oxygen consumption, and respiratory quotient *(23)*. Studies with this type of apparatus indicated that the critical moisture range in the case of wheat was associated with considerably sharper increases in respiratory activity than had been suspected. Furthermore under conditions of adequate aeration, rapid rate increases in respiration occurred in freshly wetted grain *(20)*. In the case of damp soybeans, as indicated in Figure 2, the respiration-time curve follows the typical pattern of microbiological growth, characterized by an induction period, a period of rapid increase, followed by a leveling-off, corresponding to the nature of an aging microbiological culture. This study also showed the critical effect of aeration on respiratory rate, and the fact that under nitrogen the damp seeds respired at a low and virtually linear rate.

Additional evidence that microflora appear to be the biological agency primarily responsible for the high

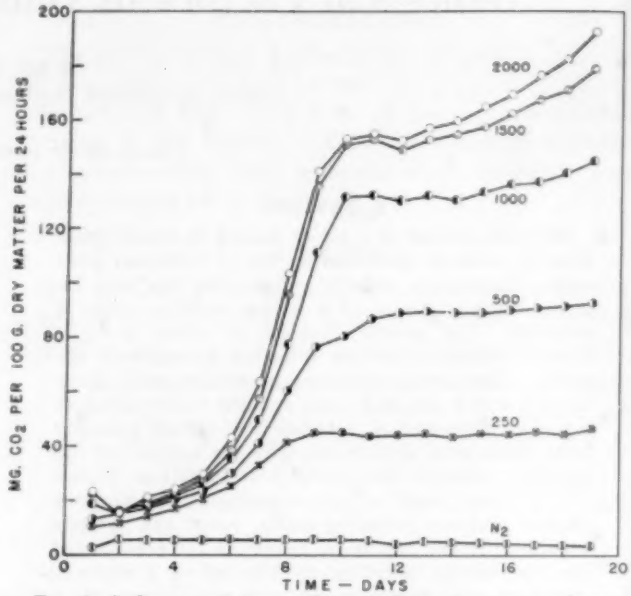

FIG. 2. Influence of time and level of aeration on respiratory activity of damp soybeans. Data of Milner and Geddes *(23)*.

respiratory activity and chemical deterioration of stored grain was obtained in studies with wheat which are summarized in Table 1.

A strong correlation exists between respiratory activity, mold population, and chemical deterioration as indicated by changes in fat acidity and in total and reducing sugars. The probability that respiration of the grain at any moisture value below that required for germination is virtually constant with time, as was shown for damp soybeans in which mold growth was inhibited by an atmosphere of nitrogen, was also suggested in studies with wheat treated with powdered sulfa drugs to inhibit the mold growth responsible for germination losses and chemical deterioration *(17)*. Thus, an indicated in Figure 3, sulfanilamide inhibited the respiration of wheat containing 20 percent moisture, in proportion to the concentration used. The low and virtually linear character of the respiration in the presence of higher quantities of the drug is of special interest. That chemical and germinative preservation of this grain occurred is shown in the data of Table 2.

Direct evidence on the relative amounts of seed and mold respiration in damp grain is difficult to obtain since no sterile, viable seeds have yet been grown. The

TABLE 1

Relationship of Moisture Content to Respiratory Rate, Mold Growth, Viability and Changes in Fat Acidity and Sugar Content of Hard Red Spring Wheat. From Milner, Christensen and Geddes (20)[c]

Moisture content		Time in respirometers	Respiratory rate [c] (final day)	Mold colonies	Respiratory quotient	Germination	Fat acidity	Total sugars	Reducing sugars
Initial	Final								
percent	*percent*	*days*	*mg./CO_2*	*per g.*	*(17th day)*	*percent*	*mg. KOH/ 10 g.*	*mg./10 g.*	*(as percent of total)*
12.3	12.0	20	0.07	500	0.60	93	35.3	252	19.0
13.6	13.1	20	0.11	100	0.85	95	35.5	263	17.9
13.8	13.7	20	0.23	100	0.91	95	35.3	237	19.8
14.5	14.3	20	0.57	400	0.89	92	37.8	252	19.8
15.4	14.6	20	2.53	4,800	0.78	91	42.3	255	20.0
16.3	16.0	20	23.4	396,000	0.91	67	66.0	248	21.8
16.8	16.4	17	20.3	209,000	0.90	88	38.6	247	20.6
18.5	19.0	17	111.0	2,275,000	0.81	37	115.0	225	21.8
20.8	22.0	17	604.9	11,300,000	0.93	14	149.7	202	27.2
25.2	30.0	17	1,724.8	37,500,000	0.99	21	140.7	184	33.7

[c] Respiratory trials were conducted at 30° C. and values obtained are expressed as mg. CO_2/100 g. dry matter per 24 hours. The values for mold count, germination, and chemical composition were determined on the wheat at the end of the respiration period.

TABLE 2

Effect of Sulfanilamide on Wheat Respiration and on the Maintenance of Wheat Viability at a Moisture Value Favorable to Mold Growth. From Milner (17)[a]

Sulfanilamide concentration	Respiratory rate [b]	Germination, percent
0	428	10
1–2,000	136	57
1–1,500	114	62
1–1,000	83	69
1– 500	52	82
1– 250	43	84

[a] Wheat samples were tested for germination after 11 days in respirometers at 30° C., at a moisture value of 20 percent. Initial germination was 94 percent.

[b] Milligrams CO_2/100 g. dry matter/24 hours, on 11th day of trial.

mold species responsible for deterioration actually occur within the seed coats of normal viable grain, and thus the use of antiseptic agents to kill or inhibit mold growth in order to study the true respiration of the seed is open to the criticism that the metabolic activity of the grain itself may be affected. In spite of these difficulties, a good indication of the relative respiratory activity of seed and mold at elevated moisture contents can be obtained with certain fungistatic chemicals. Thiourea has been shown to have a pronounced inhibitory effect on mold respiration, with little influence on seed metabolism over moderate time intervals. Thus at various moisture levels as shown in Table 3, thiourea strongly inhibited mold growth and respiration, and maintained seed germination at good levels except at moisture contents where seed germinative processes were initiated. Plotting the relationship of moisture content to respiratory activity under these conditions yielded the data of Figure 4, which probably provide a good approximation of the relationship of moisture content to the true seed respiration in wheat.

The microorganisms responsible for respiratory increases and chemical deterioration at and just above the critical moisture range include *Aspergillus glaucus*, *A. candidus*, and *A. flavus*. At a somewhat higher moisture content penicillia will proliferate. These microflora are common at least to wheat and soybeans at similar moisture levels.

HUMIDITY-GROWTH RELATIONSHIPS IN MICROFLORA

It has long been known that various maximum moisture values exist for the safe storage of different grains, and it was believed that compositional variation would account for this range in critical values. For example, the fact that flaxseed has a critical moisture

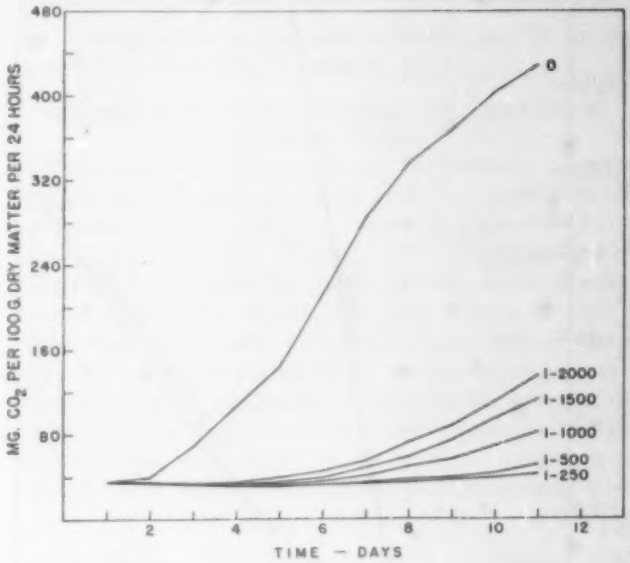

FIG. 3. Influence of various concentrations of powdered sulfanilamide on the respiratory activity of damp wheat. Data of Milner *(17)*.

of about 10.5 percent as compared to wheat at 14.6 percent was taken to indicate that only the hygroscopic constituents play a part in water retention and that the considerable oil content of flaxseed (ca. 35 percent) would reduce the hygroscopicity of that seed. This apparently logical reasoning encounters some difficulty, however, in the case of soybeans which contain 20 percent oil and yet show a critical moisture value in the same range as the starchy grains.

Resolution of this dilemma came about when it became evident that mold growth was responsible for the respiratory increases noted at critical moisture values. When these values for different grains are tabulated with reference to the relative humidity values with which these moisture levels are in hygroscopic equilibrium, the interesting fact emerges that all of the critical moisture values for different seeds are in approximate equilibrium with a common atmospheric relative humidity of about 75 percent at ordinary temperatures. Initiation of rapid respiratory activity in all seeds thus seems to depend on a water activity related to the relative humidity scale.

Several investigators have shown that over relatively short time intervals, a water activity which will produce a relative humidity of 75 percent is about minimum for the germination of mold spores at ordinary temperatures in the presence of

TABLE 3

Influence of Moisture Content and Thiourea Treatment on Respiratory Rate, Mold Growth, Fat Acidity, and Viability of Wheat. Samples Analyzed After Completion of 10-Day Respiration Trials at 30° C. From Milner, Christensen and Geddes (21)

Moisture	Respiratory rate [c]		Mold colonies per g.		Fat acidity [d]		Viability [b]	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
percent							*percent*	*percent*
14.2	0.16	0.17	5,500	5,766	14.0	18.7	96	97
16.1	2.23	0.94	5,500	4,933	15.9	20.2	94	95
17.9	100.5	6.9	10,166	5,000	50.1	14.1	26	94
21.3	461.2	42.8	5,310,000	23,700	141.2	20.4	11	93
24.3	1512.8	90.6	6,710,000	34,800	92.4	38.5	5	81
26.9	1375.4	184.4	2,580,000	10,660	87.0	21.3	8	19
30.3	2539.4	209.6	65,000,000	77,500	231.4	55.9	—	15
33.0	2267.4	291.8	88,000,000	5,575	222.3	19.2	—	10
35.5	3394.7	592.6	95,000,000	50,000	265.2	58.0	—	2

[c] Mg. CO_2 per 100 g. dry matter per 24 hours on 10th day of trial. [d] Mg. KOH per 100 g. wheat, dry basis. [b] Initial viability was 94 percent.

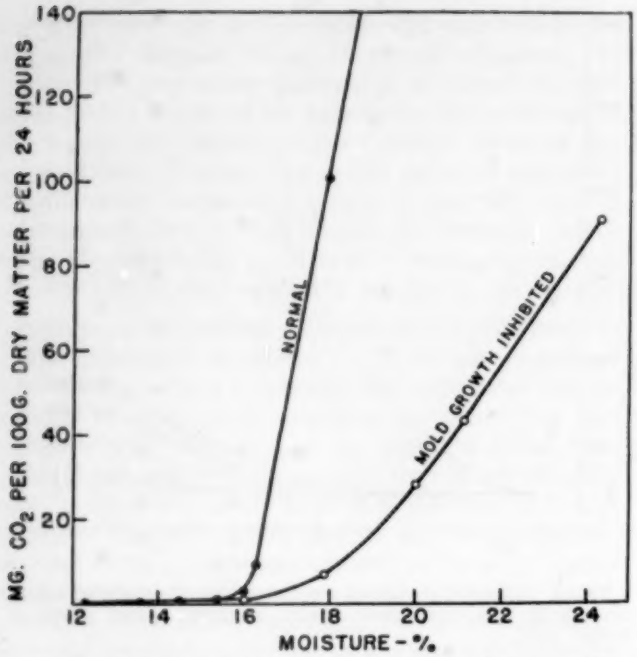

FIG. 4. Effect of mold inhibition on the respiratory activity of damp wheat. The curve marked "normal" was obtained with wheat at various moisture levels containing no mold inhibitor; the curve marked "mold growth inhibited" was yielded by wheat samples treated with thiourea. Data of Milner, Christensen and Geddes *(21)*.

adequate nutrients. A perusal of the literature leads to the conclusion that on as diversified a range of natural substrates as seeds of many species, bacon fat, meats, leather, textiles, and book-binding materials, the minimum relative humidity at which the mold spores commonly contaminating these materials will grow in time intervals of several days, is about 75 percent. This value is in hygroscopic equilibrium with the various moisture levels considered critical for these different materials *(24)*. One mycologist *(29)* has illustrated this point particularly well with the statement: "If dry samples of pure wool and pure cotton are exposed to the same (humid) atmosphere, the wool will take up approximately twice as much moisture as the cotton, and, leaving out differences due to chemical composition, the two samples will be approximately equally likely to mildew."

The dependence of microfloral growth in natural products on hydration level seems to have gone largely unexplored by industries to which these relationships are of basic importance. The information which exists on the subject indicates clearly that each species of saprophytic microflora including molds, yeasts and bacteria, has a critical hydration level for the initiation of vegetative growth. One botanist *(13)* has classified the hydration requirements of microflora as follows:

	Minimum relative humidity for germination
1. Xerophytic species	less than 80 percent
2. Mesophytic species	80 to 90 percent
3. Hydrophytic species	above 90 percent

Bacteria fall into the hydrophytic group, requiring humidities in excess of 90 percent for growth, and hence can not be expected to contribute significantly to the respiration of grain at moisture contents encountered in commercial storage. On the other hand, the species of molds found on and under the coats of normal seeds, which are responsible for the respiration and heating characteristics of stored grain, fall in the xerophytic group. Most yeasts and many fungi probably would be classified as mesophytes.

The differential hydration requirements of microflora explain the erroneous conclusion concerning the relative importance of the microorganisms contaminating grain which often have been reached when the classical approach of the bacteriologist using culture media of high water activity is employed. Thus bacteria, which actually may play no significant part in the microbiological deterioration of dry products under natural conditions, because of insufficient hydration for growth, may predominate completely over other organisms when the material is cultured on ordinary media. It is clear that one must define carefully the conditions of hydration or water availability in addition to temperature and nutrient factors before attempting to predict the microfloral species which will predominate on stored natural products. One British worker, for example, has found that mold growth with attendant lipolysis was initiated on bacon fat at 75 percent relative humidity and reached a maximum at 90 percent relative humidity; at higher humidities the molds were crowded out by the growth of yeasts and bacteria *(15)*.

The group at Iowa State *(28)* have reported on the minimum humidities required for the germination of spores of several species of molds contaminating commercial corn (Table 4). Snow, Chrichton and Wright

TABLE 4

Humidity Requirements for Mold Spore Germination. From Semeniuk, Nagel, and Gilman (28)

Mold	Relative humidity
	percent
Aspergillus amstelodami	72.5
A. candidus	72.5
A. Niger	80.0
A. flavus	82.5
Penicillium chrysogenum	85.0
P. rugolosum	87.5
P. palitans	87.5
Mucor racemosus	87.5

(30) also have reported results dealing with relationship of relative humidity to the initiation of spore germination and mold growth in a variety of natural products, particularly feeding stuffs. These workers found that over protracted time intervals (two years or more) some spore germination occurs in certain cases at humidities as low as 65 percent.

HEATING OF DAMP GRAIN

Spontaneous heating is one of the more spectacular manifestations of the respiratory activity of fungi which occurs in damp grain under conditions of storage where the heat produced is not dissipated rapidly enough. A better understanding of the special nature of spontaneous heating in stored grain has come about with the perfection of adiabatic respirometers which provide data both on heating characteristics and respiratory activity. The course of respiration and heating of damp soybeans is shown in Figure 5 *(25)*, and is probably characteristic of oil seeds generally. The first stage of heating is due to the increase of fungi under favorable aeration and temperature conditions and is accompanied by a parallel increase in respiratory rate until a temperature of 54 to 55° C. (129 to 131° F.) is reached. At this point the heating slackens and the respiration is drastically inhibited. This temperature level is highly characteristic of agricultural materials undergoing heating and is due to the fact that the fungi responsible for the heating are killed by the accumulated heat which they themselves have produced. Under conditions of very high moisture where thermophilic bacteria may grow, this initial biological heating stage may proceed to 68 to 70° C. (154 to 158° F.).

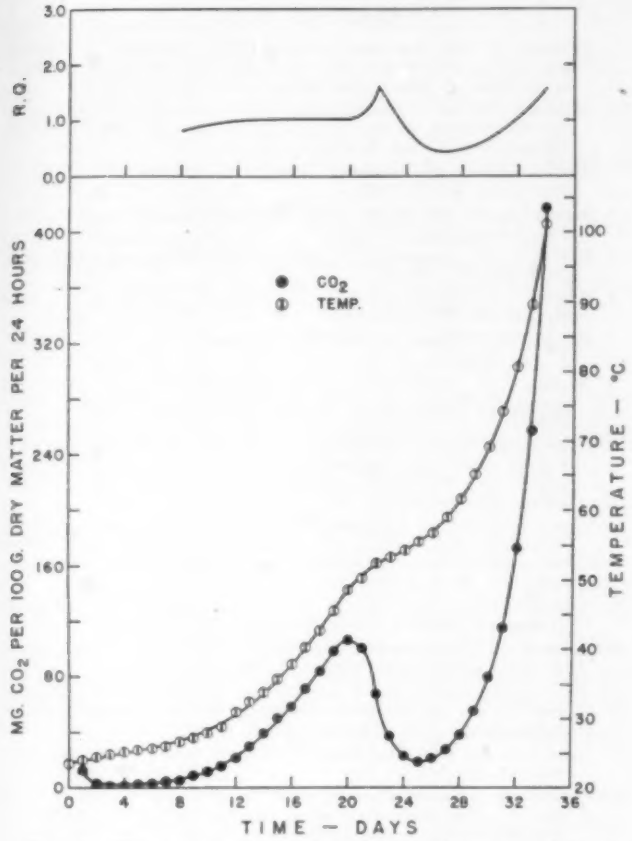

FIG. 5. The course of heating and respiratory activity in ordinary soybeans under conditions close to adiabatic. Data of Milner and Geddes *(25)*.

A second heating stage following inhibition of the biological agencies may occur which apparently carries the heating and carbon dioxide production to extremely high levels. This stage is due to chemical oxidation and is nonbiological in character. Changes in the chemical composition of soybeans due to the elevated temperatures caused by spontaneous heating are shown in Table 5, and include an increase in non-protein nitrogen and reducing sugars and a decrease in seed germination and fat extractable by ordinary solvents. The extent of fungal infection increases to a maximum in the temperature range of 45 to 50° C. (113 to 122° F.), but the heating material becomes virtually sterile by the time 60° C. (140° F.) is attained *(25)*.

POSSIBILITIES FOR CHEMICAL PRESERVATION OF DAMP GRAIN

The realization that fungi are the primary agencies causing deterioration in damp grain has prompted investigation of the utility of chemical preservatives to control grain spoilage. The utility of sulfanilamide and thiourea for suppressing mold growth in respiration studies with wheat already has been referred to *(17)*. More than 100 organic compounds have been screened *(21)* for effectiveness as mold inhibitors and several were assayed as respiratory inhibitors as indicated in Figure 6. The compounds 8-hydroxyquinoline sulfate and thiourea were shown to be excellent inhibitors of respiration in damp wheat, while p-aminobenzoic acid, a non-toxic metabolite, appeared also to be rather

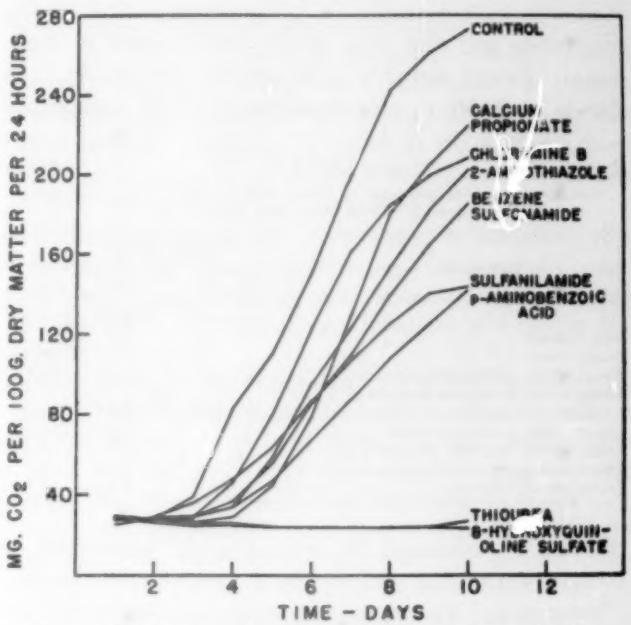

FIG. 6. Utility of various organic compounds as respiratory inhibitors in damp wheat. Data of Milner, Christensen, and Geddes *(21)*.

TABLE 5

Influence of Spontaneous Heating on Chemical Composition, Mold Infection, and Germination of Soybeans Containing 22.8 Percent Moisture. Milner and Geddes (25)

Days	Temp.	Chemical analysis							Seeds infected with fungi [2]	Germination
		Crude protein (N x 6.25 dry basis)	Non-protein nitrogen	Sugars				Crude ether extract (dry basis)		
				Total [1]	Non-reducing as sucrose	Reducing as glucose	Reducing as percent of total			
	°C.	*percent*	*mg./10 g.*	*mg./10 g.*	*mg./10 g.*	*mg./10 g.*	*percent*	*percent*	*percent*	*percent*
0	24.4	35.4	22.4	444	400	44	10.0	21.6	3	93
3	29.4	35.4	21.2	410	378	32	7.8	19.5	11	73
5	37.1	35.8	21.2	405	373	32	6.9	20.2	16	52
7	44.4	35.1	22.2	378	347	31	8.1	20.2	27	30
8	49.3	35.2	23.6	433	382	51	11.8	20.3	64	0
10	53.7	35.4	28.2	417	334	83	19.9	19.4	40	0
12	55.1	35.7	34.7	491	245	246	50.1	15.3	14	0
13	55.2	35.8	32.4	438	251	187	42.6	14.9	13	0
15	59.7	36.1	32.3	443	226	217	48.9	13.6	9	0
17	67.3	36.3	35.7	432	172	260	60.3	12.0	0	0
19	77.0	36.6	35.0	291	27	264	90.8	10.5	0	0

[1] Sum of sucrose and glucose. [2] The majority of the fungi were Aspergillus spp.

effective. Snow and Watts *(31)* recommend the treatment of certain feedstuffs with sulfa drugs to inhibit their spoilage.

Great many organic compounds have been evaluated as inhibitors of heating in stored flaxseed *(1)*. Treatment of the seed with propylene glycol dipropionate and bischloromethyl xylene are useful to control heating *(2)*. Treatment of damp stored rice with acetic acid and sodium bisulfite was found to be effective in controlling the heating of that crop *(3)*.

Several other chemical treatments have been investigated with reference to their utility in inhibiting changes in damp wheat which would affect its quality from the point of view of commercial grade and bread production purposes *(16)*. Treatments applied included carbon tetrachloride, propylene oxide, a 1-1 mixture of carbon tetrachloride and propylene oxide, ethylene chlorohydrin, piperonyl cyclonene, and thiourea. All of these chemicals have been shown to be effective in reducing respiration and rate of fat acidity development in damp wheat. Stored samples were aerated periodically. As shown in Table 6, all treatments reduced commercial

TABLE 6

Graded Commercial Damage in Damp Wheat After Various Storage Intervals. From Matz and Milner (16)

Treatment	Weeks storage			
	4	8	12	16
		graded damage		*percent*
Dry control	0.0	0.0	0.0	0.0
Damp wheat	0.0	1.0	16.0	18.0
Damp wheat, carbon tetrachloride	1.3	2.6	4.8	10.0
Damp wheat, 50 percent propylene oxide in CCl_4	1.0	2.6	5.0	6.5
Damp wheat, propylene oxide	0.6	1.0	2.0	8.0
Damp wheat, ethylene chlorohydrin	1.4	3.4	7.0	15.0
Damp wheat, piperonyl cyclonene	0	1.0	8.5	8.5
Damp wheat, thiourea	trace	1.2	4.0	6.6

damage as estimated by federal grain inspectors, in comparison to that suffered by a damp control sample. Thiourea and the mixture of propylene oxide and carbon tetrachloride were the most effective in this regard. Flours milled from these wheats showed a marked tendency towards increased mixing time of the doughs. This characteristic was common to all such dough regardless of treatment.

When the flours milled from these wheats were baked after 16 weeks of storage, it was found that the material obtained from the damp samples regardless of treatment exhibited undesirable gray crumb color and musty odor. All treatments showed minor to considerable increases in specific volume of loaves in comparison to those from flours obtained from damp untreated control samples; in a few cases the specific volume of certain treated samples was greater than that of the undamaged dry wheat. An improving effect was evident in the case of the mixture of propylene oxide and carbon tetrachloride. Thiourea was deleterious in this regard. A notable characteristic of the flours from all the damp samples was a marked decrease in response to the oxidizing improver, potassium bromate.

While these results are not entirely conclusive, they do indicate that further research into the use of preservative chemicals for inhibiting the spoilage of damp grain is to be encouraged.

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Several Practical Quality Control Measures[a]

HATTON B. ROGERS, JR.
Phillips Packing Company, Inc., Cambridge, Md.

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Some quality control measures in the manufacture of tomato products, of "beans and franks" and in chicken noodle soup are discussed as illustrations of what systematic approach to such problems may accomplish. The need for definite responsibility and authority for those in charge of quality control is emphasized.

As a starting point in this discussion it would perhaps be wise to define just what is meant by "practical quality control." According to Webster, the word "practical" means "pertaining to or governed by actual use and experience as contrasted with ideals and speculations." "Control" is defined as "exercising direction or restraining or governing influence." And finally "quality' is defined as "condition as determined by its degree of goodness and relative excellence." Therefore, assembling all of these definitions we arrive at the following: practical quality control is "governing by direction and actual experience a degree of excellence in a product."

I know of no better statement regarding quality control than that given by Hall *(1)*: "Some people think of quality control systems as packages which can be purchased by hiring food technologists. Some people think of the quality control departments as a mill stone to be hanged on the neck of a reluctant production department to watch and make it pack quality. Others as mentioned previously think they are fine,—everyone is talking quality and a quality control department will impress the buyers. The expense will be charged to sales."

From this statement it is evident that quality control is sometimes misused and misunderstood. It is possible that quality control has fallen short of what has been expected of it in some cases. This is primarily due to two things: First, the quality control department may ignore the *practical* aspect of a given problem, and second, top management may fail to delegate authority which is commensurate with responsibility. Either of these conditions is regrettable, but one, or both, exist in some organizations and must be recognized.

Before a quality control department may become truly effective, there must be decisions as to where the responsibility and the authority of this section of management begins and ends. Basically this group must be autonomous in its operation, reporting directly to top management. This is for ease of operation since only top management is able to make the decisions respecting changes required to maintain quality at the level established by company policy. Autonomy permits proper interpretation of technical information and experimental results and avoids transmission of incomplete or inaccurate reports. This allows top management to base its decisions on accurate data, comprehensive in scope and brief in form.

Before giving several illustrations of the application of practical quality control, I would like to point out that these will not be, and are not intended to be, startling applications. They are intended to be of a basic nature. The reason for this choice will be apparent to those who have been charged with the responsibility of launching a quality control department. Often a long period of time may elapse before quality control becomes completely effective. In the eyes of many, even those who desire it most, it must put in its apprenticeship on the simple and immediate problems before sailing into the more fundamental problems and the deeper waters of complex overall control. There is much to be said for a slow start. It permits the personnel to examine all of the operations at first hand and form their opinions as to the relative merit of different procedures, unhampered by past association or too much immediate responsibility.

I shall not discuss the construction or equipping of a laboratory, the main tool of the quality control department, since it has been covered at some length in many discussions of which that by Alexander and Braun *(2)* will be noted here.

Let us begin our discussion of practical quality control by considering one of the basic problems confronting the packer of specialty items. In the production of "pork and beans," "vegetable soup," "vegetable beef soup," and "beans and franks," where the tomato pulp plays a very important role in the color and flavor of the product, it is necessary to maintain the desired color of the sauce or soup stock at a uniform level throughout the entire pack. This poses the serious problem of uniformity of color. In the east and midwest, but more particularly in the coastal regions, this problem has become more acute from year to year. Examination of the records of tomato loads will show that the tomatoes used in the manufacture of pulp or puree come from many locations and that the color varies in the individual growing areas. Therefore, in order to obtain the best color, it is desirable to select loads having the best, and most uniform color. To establish a system of grading by color which covers the best and the worst colors which might be produced in the course of a year and several color grades between the two extremes, we have developed a scale type color chart for use in the grading of tomato puree. This set of color standards, which is easily reproduced, is now used to grade the final product. If the specific gravity is controlled and the only remaining, troublesome variable, color is graded, we have a controlled product.

By a system of numbering and grading it is possible to warehouse and utilize all of the varying shades of tomato puree in the following manner:

[a] Presented before the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 23, 1950.

1. As soon as the pack is finished the percentage of each of the five major color divisions is determined and these figures are used as a basis for calculating potential utilization.
2. The tomato puree is then tested by combining some few, or all, of the five color grades, as discretion dictates, to determine just what formula will achieve the prescribed color in the final product.
3. Assume that, on the basis of color classification for a season's pack, the following percentages of color are obtained for the pulp stored in the warehouse:
   Color Number 1—10 percent
   Color Number 2—45 percent
   Color Number 3—30 percent
   Color Number 4—10 percent
   Color Number 5— 5 percent
   The figures may appear a little lopsided but it should be remembered that these are arbitrary standards and are established so that the desired color falls in the lower part of the number 2 range.
4. The desired color for the finished product falls also in the lower part of the Number 2 range, and it is possible, therefore, to use this color and also that of color Number 1 to raise the color of the puree graded Number 3, Number 4, and Number 5. The first tests conducted in the laboratory, under controlled conditions, will yield a rough approximation of the percentage of each color which may be used to achieve the desired result. Thus, in this case, it might be found that 85 percent of the Number 2, plus 12 percent of the Number 3, plus 2 percent of the Number 4, and 1 percent of the Number 5 would give the desired color.
5. By using this experimentally determined ratio a standard color can be maintained and all of the pulp of low color grades may be used simultaneously. This insures against having a quantity of puree of low color grade remaining at the end of the year's pack.

The question might well be asked, "Would it not be far better to grade tomatoes for color as they arrive and reject those which do not come up to a very critical standard?" The answer to this is emphatically yes,—if it can be done practically and economically. However, if large quantities of tomatoes are to be used, the color of even carefully selected loads will still be somewhat variable.

The grading system, although it requires some additional time on the part of all, and more space in the warehouse, is a practical solution to the utilization of the tomato puree produced.

In the foregoing illustration, control was accomplished by devising and using a new type system for a particular product. The next two illustrations are principally concerned with the use of machines to achieve control of the product.

At the start of production for any product there are usually several bugs to be worked out of the operation. Some of these are simple production problems which cannot conceivably come under the jurisdiction of the quality control department. However, in many instances, these small problems attain gigantic proportions when it is discovered that they bring the final product into a position of questioned excellence by reason of a deficiency of a supposedly minor nature.

An illustration of this point may be taken from the control of quality in a specialty product known as "beans and franks." This product was originally developed by the Army to supply variation in the meat items of the old "C" ration. However, under the specifications prepared at that time the frankfurters were sliced into small pieces, and these were mixed as well as possible with the beans and then filled into the cans. This mixing and filling method resulted in a product with little or no uniformity in the number of pieces of franks found in individual cans, even though the average number for a hundred cans showed uniformity. Thus, in the conversion of this product from one for the Army to one for the housewife, the problem confronted was the elimination of the possibility of obtaining a can full of beans one time and a can full of franks the next. Many methods were tried in efforts to eliminate this difficulty, the first of which was placing a given number of pieces in each can by hand. Obviously this was slow, expensive, and subject to unavoidable human error. The next remedial efforts consisted of using various types of commercial fillers which were available, and these met the filling problem with varying degrees of success. Some filled well but damaged the pieces, while others delivered varying amounts to the cans with little improvement over the original filling method. At this point a machine was developed which would cut uniform pieces of the sausage and deliver a given number of pieces of a given weight to 160 cans per minute. The variation of fill obtained with this specially designed machine is so small that the filling problem is no longer an uncontrolled quality factor.

Another problem of a somewhat similar nature was encountered in the production of "chicken noodle soup." However, there were three variables which had to be controlled in this case. These variables were as follows: the amount of chicken fat per can, the amount of noodles per can, and the amount of diced cooked chicken per can. In the production of this condensed soup the chicken is simmered for a period of time in just enough water to cover it well. This cooking provides the broth for the soup, and the resultant broth contains a large percentage of the fat present in the fowl. Since the amount of fat in any given lot of fowl will vary considerably, the amount present in the finished soup will vary in a like manner unless controlled by some method. Several methods of determining the fat content of the broth were tried, but the results were poor because of sampling variations. The most practical method of overcoming the fat variation appeared to be complete removal of the fat from the broth. This was done prior to making the final batch of soup stock. When the batch was finally made the amount of fat required by the formula was added, thus providing the desired uniformity. By using agitation the fat was kept in finely divided particles and each can received its pro rata share.

The second variable in this operation had two parts. The first was the lack of uniformity of the noodles produced commercially under specifications, and the second, differences in the weight and volume of the noodles in each can. After considerable experimentation the conclusion was reached that it would be far better to produce noodles to a set specification in the plant rather than use commercial noodles. By using specially designed commercial noodle making equipment and a filler of commercial design which was modi-

fied in order to do the job, the required amount of noodles was filled into the can, giving the uniformity which was necessary for the product.

The final variable, that of mechanically placing a required amount of diced cooked chicken in each can, required considerable experimentation. Several machines were designed and used for short periods of time. A final solution was obtained by a machine of entirely different design which eliminated clinging of the cooked chicken to the integral parts of the machine. The machine is simple in construction, easy to clean, and can be adjusted for variations in fill.

With all three of these machines placed in a line the production of this soup was accurately controlled, and all of the variables which were first encountered were reduced to variances within good commercial practice.

The foregoing illustrations have concerned machines and the position that they are able to occupy in the scheme of quality control. The next illustration is one equally basic and very high on the list of importance as far as the peace of mind of the canner is concerned.

As a matter of routine, samples of the pack should be taken each day and incubated at either 98° F. (37.0° C.) or 135° F. (55° C.), or both, if deemed advisable for the detection of spoilage. There are several methods of choosing samples for incubation which will depend on the product being packed and the type line being used. These methods have been covered at length by Thompson *(3)*, Cameron *(4)* and Pearce *(5)*. Spoilage of incubation samples, regardless of the method of collection, will forewarn the canner of impending trouble. Accurate records should be kept of all incubation samples and the results carefully tabulated. In the case of spoilage full information will be available.

The foregoing illustrations have been used to assist in telling the story of practical quality control rather than to indicate new methods or tests. These measures may be in use in modified form by many organizations today, and the main reason for mentioning them here is to bring to your attention the fact that procedures such as these can give comprehensive control over all products.

A more intangible result of quality control procedures, properly established as a company policy, is that they acquaint the factory personnel with the sincere desire of the company to pack products of uniformly good quality.

This feeling, call it "quality mindedness" if you will, is difficult to build in employees and once attained will be the first bulwark of defense against a lowering of quality due to the human error factor. Though this feeling is difficult to obtain, it is easily lost if, for some reason, the quality standard is discarded. For only chaos can exist in the minds of those who are told one minute to keep the standard up and the next are told to lower it considerably. This feeling of "quality mindedness" on the part of all helps immeasurably in the execution of the duties of quality control and can never be replaced by any test. If the quality control department can look for the basic facts, those variables which stand out as needing control, and then proceed to bring them in line by some simple method, a great step forward has been taken in its established sphere. For quality control is conceived in thought but born of service.

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Survival of Microorganisms in Concentrated Orange Juice[a]

L. W. FAVILLE,[b] E. C. HILL,[c] and E. C. PARISH[b]

(Received for publication, May 29, 1950)

Observations on 13 packs of 42° Brix orange concentrate indicate that bacteria, rather than yeasts, are the predominating organisms present. These bacteria die rapidly in storage. Tests with pure cultures of yeasts and bacteria indicate that although some of these are able to multiply in single-strength orange juice at ordinary temperatures, the bacteria at least are rapidly killed during the storage of 42° Brix concentrate at similar temperatures.

At the present time there is a relatively small amount of information available regarding the microflora of citrus juices. This situation is probably best explained by the fact that an efficient pasteurization process together with the acidic nature of the raw material has practically precluded any possibility of spoilage in canned citrus juices. However, the recent development of unpasteurized, frozen citrus concentrates has created a need for information regarding the types of microorganisms which are present in citrus juices together with a knowledge of the fate of these organisms during the evaporation process and subsequent storage at subfreezing temperatures.

Nearly all of the previous work which has been concerned with the survival of microorganisms in citrus juices has dealt with single-strength juices. Beard and Cleary *(1)* demonstrated that a low temperature [—4° C. (24.8° F.)] will allow the survival of intestinal pathogens for dangerous periods of time in orange

[a] Presented at the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 24, 1950.

[b] Citrus Experiment Station, University of Florida.
[c] Florida Citrus Commission.

juice at hydrogen-ion concentrations which would be highly bactericidal at ordinary temperatures.

Schrader and Johnson *(4)* studied the survival of *Escherichia coli, Lactobacillus acidophilus* and a member of the *Bacillus subtilis* group in orange juice at several temperatures and concluded that the death rate of these organisms was most rapid at 37° C. (98.6° F.), was slower at 25° C. (77° F.) and was still slower at —12° C. (10.4° F.). They further concluded that organisms of the *E. coli* type could not survive longer than two weeks in frozen orange juice.

At least two factors should be considered when comparing the survival of microorganisms in concentrate with survival in single-strength juices. In the first place, the concentration of orange juice to a 42° Brix concentrate results in an approximate four-fold increase in acidity. On the other hand, a corresponding increase in soluble solids, mostly sugars, should enhance the possibility of organisms surviving during storage at low temperatures. This fact is well illustrated by the work of McFarlane *(2)* who found that *E. coli* and an unidentitfied *Torula* strain survived better in an orange juice containing 40 to 50 percent added sucrose than in unsweetened juices when stored at —17.8° C. (0° F.).

More recently Patrick *(3)* found that storage of orange concentrates at —17.8° C. for 198 days resulted in a reduction in the number of spoilage organisms present and that yeasts predominated among the surviving organisms.

EXPERIMENTAL

A representative number (48) of 6 oz. cans of 42° Brix frozen orange concentrate from each of 13 separate batches were stored at —17.8° C. and examined bacteriologically at intervals of one month for a period of eight months. Duplicate cans were removed from storage at each sampling and plated on differential media for total microorganisms, yeasts and molds, acid-forming bacteria and peptonizing bacteria.

Total plate counts were determined by using dextrose agar (Difco) adjusted to pH 7.3 and yeasts and molds by the use of potato dextrose agar (Difco) acidified to pH 3.5 by the addition of a 10 percent tartaric acid solution. Acid-formers were determined by plating on a medium consisting of 1 percent tryptone, 0.5 percent yeast extract, 0.3 percent beef extract, 0.1 percent dextrose, 0.1 percent K_2HPO_4, 1.5 percent agar and 0.002 percent brom cresol purple indicator. Peptonizers were determined by the use of nutrient agar to which 5 percent sterile skim milk had been added.

These media, with the exception of acidified potato dextrose agar, were incubated for 48 hours at 30° C. (86° F.) before counting. Yeast and mold counts were made after a 5-day incubation at room temperature.

An estimate of the number of microorganisms (bacteria, yeasts and molds) capable of growing at refrigerator temperature was made by plating on dextrose agar and incubating the plates at 4.4° C. (40° F.) for a period of 30 days before counting. These organisms although designated as "psychrophiles," were not obligately psychrophilic and grew well at 30° C.

In order to compare the survival of microorganisms in concentrates with their survival in single-strength juices, pure cultures of selected microorganisms were used to inoculate 100-ml. aliquots of a 42° Brix orange concentrate (pH 3.5) and a 10.5° Brix orange juice (pH 3.68) prepared by reconstituting one volume of concentrate with three volumes of water. These juices were sterilized by steaming for 15 minutes in an Arnold sterilizer. After inoculation with 1 ml. of a 24-hour broth culture of the test organisms, the flasks were incubated at 30° C. and samples removed at hourly intervals and plated on appropriate media.

The organisms used for this study were *Leuconostoc mesenteroides*, an unidentified *Rhodotorula* and a Gram-negative bacterium, capable of growing in single-strength orange juice, which was identified as most probably belonging to the genus *Achromobacter*. In addition to these organisms, all of which had been isolated from citrus juices, a strain of *E. coli* was also used.

A similar experiment was conducted to determine the effect of temperature on the survival of these organisms in a 42° Brix orange concentrate. Temperatures of 30° C. and 40° C. were obtained in a thermostatically controlled water bath and 3° C. in a standard refrigerator.

In both experiments, violet red bile agar (Difco) was used as a plating medium for *E. coli*, the dextrose-tryptone-yeast extract agar previously described for *L. mesenteroides*, and dextrose agar (Difco) for the *Rhodotorula* and *Achromobacter* strains.

RESULTS AND DISCUSSION

Contrary to one of the popular conceptions existing in the citrus industry, yeasts do not in all cases appear to be the predominating organisms present in citrus juices. Figure 1 represents an average of the counts

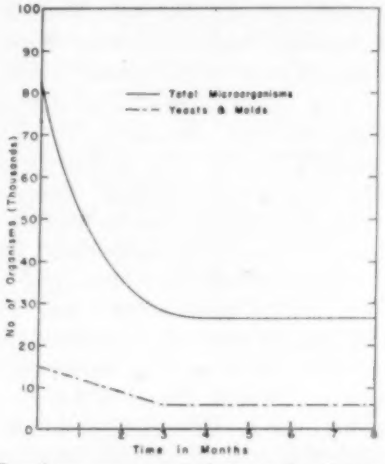

FIG. 1. Survivor curves of (a) total microorganisms and (b) yeasts and molds in 42° Brix orange concentrate stored at —17.8° C.

obtained from 13 separate batches of concentrate and indicates that there were originally present in these samples at least five times as many organisms capable of growing on a medium having a pH value of 7.3 as there were organisms which could grow at pH 3.5. Since most of the yeasts in citrus juices are able to multiply at pH 3.5, the difference between the two counts is no doubt largely due to the presence of a large number of bacteria. Also, the sharp decrease in total count during the first three months of storage as compared to the gradual reduction in the numbers of yeasts and molds indicates that the majority of the bacteria present in frozen concentrate die more rapidly during storage at sub-freezing temperatures than do the yeasts.

Early in this study it was noted that the five packs of concentrate secured from commercial plants had a much higher initial count than the remaining eight experimental packs which had been processed in the pilot plant located at the Citrus Experiment Station. Also it was evident that there was a much sharper decrease in the number of viable organisms during the first few months of storage in the commercial packs. For these reasons, the results obtained from the concentrates from these two sources are presented separately in Figure 2. Although the total number of

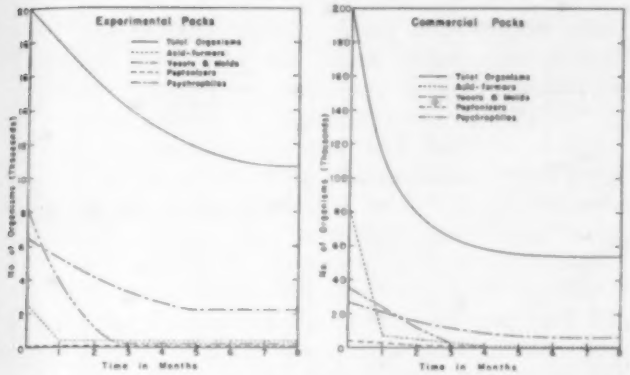

FIG. 2. Comparison of survivor curves of total microorganisms, yeasts and molds, acid-formers, peptonizers and psychrophiles in commercial and experimental packs of 42° Brix orange concentrate stored at —17.8° C.

microorganisms in the commercial concentrate was ten times greater than in the experimental concentrate, almost 50 percent had died during the first month of storage as compared with an approximate 10 percent decrease in the experimental packs during the same period. Coincident with this rapid reduction in total count during the first month of storage of commercial concentrate is a similar decrease in the number of acid-formers. These organisms, which constituted nearly 40 percent of the total count, were predominately members of the genus *Leuconostoc*. Of a total of 54 acid-forming organisms which were isolated and identified, 31 proved to be *L. mesenteroides*, 13 *L. citrovorum* and 10 assorted strains which were not identified. Similarly, of a total of 45 bacterial cultures isolated from dextrose agar plates incubated at icebox temperature for one month, 38 were identified as *L. mesenteroides*.

Casein-digesting organisms, principally *Bacillus cereus* and *Bacillus megatherium*, were not present in sufficient numbers to be significant

The remainder of this study deals with the survival of pure cultures of bacteria and yeasts in citrus products at temperatures above the freezing point of concentrate. While probably representing only a relatively small percentage of the species found in citrus juices, the organisms used in these experiments are nevertheless significant. For example, *L. mesenteroides*, one of the lactic acid bacteria, has a limiting pH very nearly that of orange juice and, for that reason, might be expected to be a potential spoilage organism. *E. coli*, while not isolated from citrus juices, is representative of the intestinal group of bacteria and is therefore of considerable sanitary significance. The strain of *Rhodotorula* was isolated from orange juice as was the strain of *Achromobacter* which was used. This latter organism is of particular significance by virtue of its ability to multiply in an orange juice having a pH of 3.67.

Figure 3, in which survivor curves of these organisms in a 10.5° Brix orange juice and a 42° Brix orange concentrate are compared, clearly illustrates the marked difference in the bactericidal activities of concentrate and single-strength juice. Of the four organisms tested, only *E. coli* showed a significant decrease in the 10.5° Brix juice during an incubation period of seven hours at 30° C. The *Achromobacter* and yeast strains, however, both showed a ten-fold increase in numbers during the same period while *L. mesenteroides* was unchanged. In concentrate, however, an entirely different situation exists. *E. coli*, *L. mesenteroides* and the *Achromobacter* strain were rapidly destroyed, all being reduced from an initial count of over 10^6 organisms per ml. to less than 10^2 organisms per ml. during the period of incubation. The yeast alone was unaffected by this treatment, neither dying nor multiplying.

From these results, it is apparent that raw orange juice cannot be safely held for a period longer than a few hours at ordinary temperatures without growth of some of the microorganisms present. Concentrate, on the other hand, would probably be stable over a reasonable period of time.

The effect of holding temperature on the survival of these organisms is presented graphically in Figure 4. From these graphs it is clearly seen that temperature is a very important factor in the die-off of bacteria in concentrated citrus juices. Yeasts, however, do not appear to be affected appreciably at any temperature. Of the three bacterial species used in this experiment, only *E. coli* was significantly reduced in numbers during the seven-hour incubation period at 3° C., decreasing from an original count of nearly 10^7 organisms per ml. to less than 10^5 organisms per ml. At 30° C. and 40° C., however, the reduction in numbers of *E. coli* was very rapid and, in the case of the latter

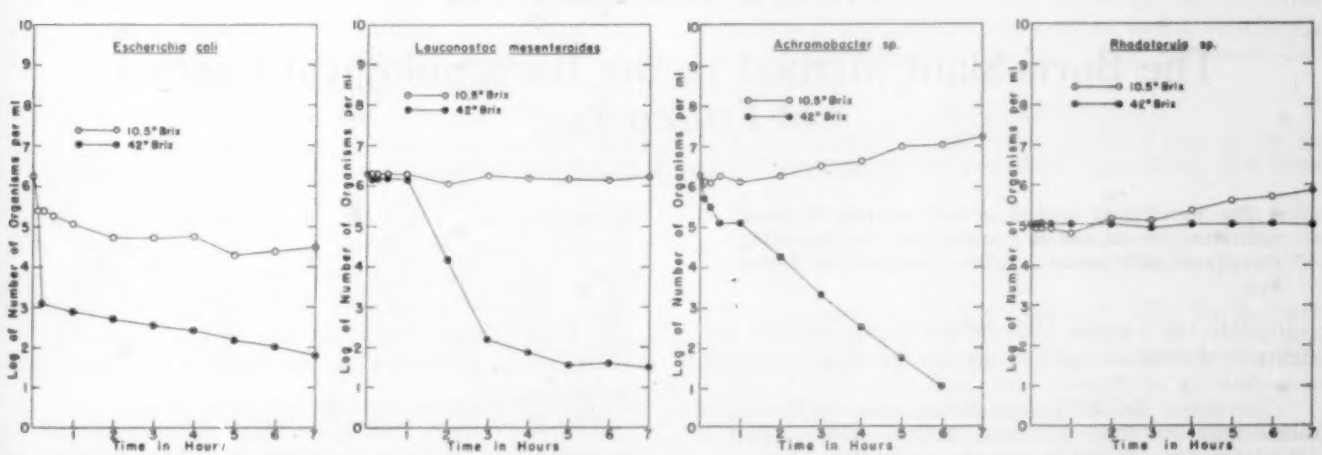

FIG. 3. Survivor curves of selected microorganisms in (a) single-strength orange juice and (b) 42° Brix orange concentrate at 30° C.

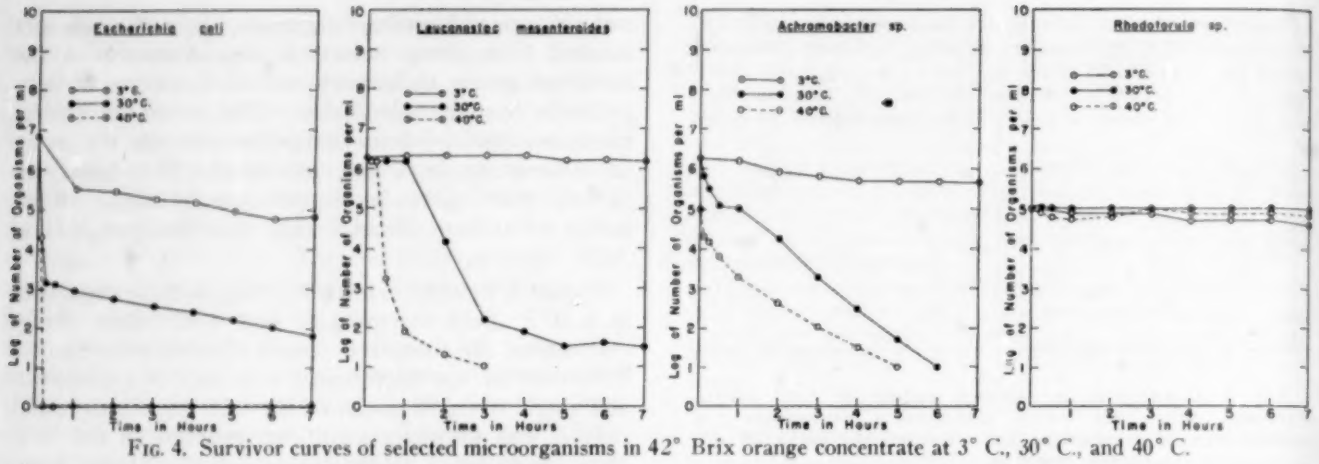

FIG. 4. Survivor curves of selected microorganisms in 42° Brix orange concentrate at 3° C., 30° C., and 40° C.

temperature, the organisms could not be demonstrated by plating procedures after 15 minutes exposure.

Although not appreciably affected by incubation at refrigerator temperature in concentrate, *L. mesenteroides* and the strain of *Rhodotorula* were rapidly killed at the higher temperatures. Both of these organisms were reduced in numbers from more than 10^6 cells per ml. at the beginning of the experiment to less than 10 per ml. after four or five hours.

From the results of these experiments it appears that there is very little danger of spoilage occurring during any ordinary process providing that no attempt is made to hold a single-strength juice at elevated temperatures for periods longer than a couple of hours. From the standpoint of spoilage due to *microbiological activities alone*, attempts to maintain low temperatures in the product throughout the process by the use of cold-wall tanks and slush freezing probably serve only to preserve those organisms already present in the juice.

SUMMARY

Based on observations made on 13 packs of 42° Brix orange concentrate stored at —17.8° C. (0° F.) bacteria, rather than yeasts, appeared to be the predominating organisms present. These bacteria, a large portion of which were identified as belonging to the genus *Leuconostoc*, died off rapidly in storage.

Yeasts, on the basis of differential plating media used in this study, comprised only about one-fifth of the total count obtained.

On the basis of results obtained by studying the survival of pure cultures of yeasts and bacteria in single-strength orange juice and 42° Brix orange concentrate, it is concluded that although yeasts and certain bacteria are able to multiply in an unconcentrated orange juice at ordinary temperatures, the bacteria at least are rapidly killed in a 42° Brix orange concentrate at similar temperatures. It is probable that most of the organisms which die during the first two or three months storage at —17.8° C. would have been killed during an exposure of several hours at ordinary temperatures.

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Notes and Letters

The Burri Slant Method in the Bacteriological Control of Frozen Egg[a]

C. K. JOHNS AND J. G. DESMARAIS

The Burri slant method proved valuable in plant sanitation control, and as a sorting test for estimating compliance with bacteria count standards for frozen egg.

In 1947 the Canada Department of Agriculture prescribed regulations concerning the grading, marking, inspection and shipment of frozen egg *(2)*. While bacteria count standards were not introduced until 1948, efforts were made to instruct plant operators in approved plant procedures, particularly since sanitation and bacteria were unfamiliar concepts to some of them. In this work extensive use was made of the Burri slant technique *(1)*.

The Burri slant technique had previously proved its usefulness in the control of plant sanitation in Canadian

[a] Contribution No. 307 Journal Series, from the Division of Bacteriology and Dairy Research, Science Service, Dept. of Agriculture, Ottawa, Canada.

egg drying plants *(4, 5)*. Along with regular plant sanitation surveys by the senior author, the resident inspectors were trained in the use of this technique for the routine control of breaking room operations. In addition to inoculating slants with loopfuls of liquid egg, wash water, tray drip, etc., swabbings were made of equipment surfaces and the swabs rubbed over the surface of slants. This technique was found to be extremely valuable in impressing upon plant personnel the importance of various procedures, since the Burri slants could be inoculated in their presence, then left in the plant to incubate at room temperature for a few days so that they could observe the growth of the bacterial colonies. Even though the Burri slant method may be less accurate than the plate count method *(3)*, plant personnel are much more impressed with the results of such a test than with one where samples are taken away to the laboratory and a report comes back several days later. At a number of plants, keen interest was shown in this technique by the workers as well as the management, and it is felt that the use of this technique had something to do with the excellent record achieved by Canadian egg dryers. By means of this technique, the effect of unsatisfactory equipment and methods could readily be demonstrated, so that co-operation in undertaking remedial measures was readily obtained.

When in 1948 bacteria count specifications were put into force for Grade A frozen egg, a number of difficulties were encountered. Many breaking plants were located at points where no bacteriological laboratory existed. Shipment to central points was also complicated by the unavailability of dry ice at many smaller towns. A further consideration was the cost of analysis by the plate count method.

As a result of educational work with the plant operators during 1947 and 1948, the average level of bacteria counts for frozen egg was reduced appreciably. It appeared, therefore, that a considerable saving could be made by employing a simple screening test to pick out high count samples which could then be analyzed by the plate count method. For this purpose the Burri slant technique again appeared to be the most suitable. However, before officially adopting it for this purpose, parallel counts by both Burri slant and plating methods were made in this laboratory on 250 samples of frozen whole egg representing a wide range of conditions of processing. From the results presented in Figure 1, it will be seen that with the majority of samples the counts by the Burri slant method were in reasonably good agreement with those by the plate count, although with the higher count samples, the overcrowding of colonies on the Burri slants resulted in relatively lower counts by this method. The arithmetic averages (150,000 for the Burri slant and 220,000 for the plate count) are indicated by broken lines in Figure 1. However, of the samples exceeding the plate count limit of 2,500,000 per g. for Grade A,[b] only three gave counts of less than

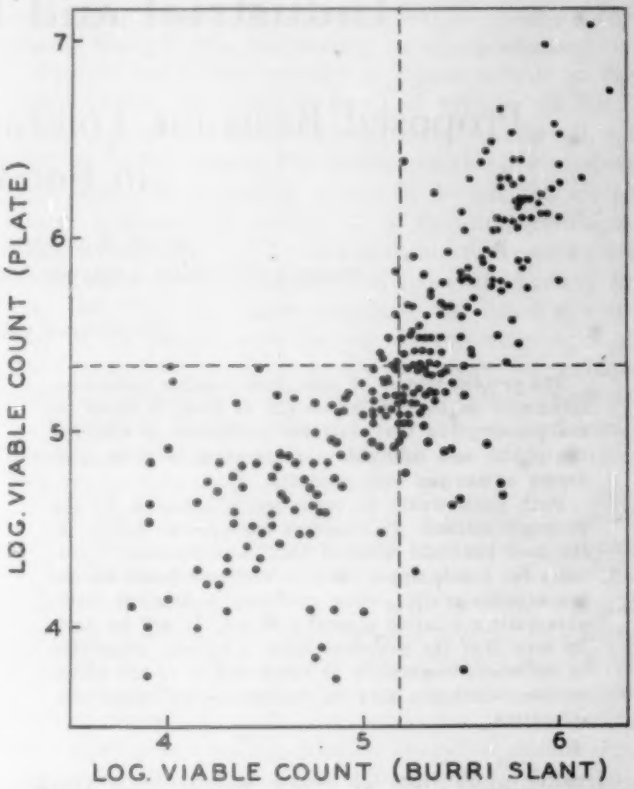

FIG. 1. Comparative counts on 250 samples of frozen whole egg by Burri slant and plate count methods.

500,000 (250 colonies) by the Burri slant method. It was decided, therefore, that the latter method was suitable for use by the inspectors as a screening test. If the Burri count per g. was less than 500,000, it could be assumed that the plate count would not exceed 2½ million; if more than 500,000, a duplicate sample would be sent to the appropriate laboratory for a plate count. On 634 Burri slant determinations made by the inspectors in 1949, only 12.8 percent exceeded the 500,000 per g. limit and had to be re-analyzed by the plate count method.

The Burri slant method has shown itself to be a most useful tool in controlling plant sanitation in the egg industry. It is inexpensive, the technique is easily acquired and it has the supreme advantage that the entire procedure can be carried out on the spot. In all probability, it could be used to advantage in other branches of the food industry as an aid in developing sanitation-consciousness among plant personnel and management.

[b] In the light of the improvement shown since 1947, this preliminary standard is much too lenient. An early downward revision is anticipated.

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Industrial and Legal Viewpoints

Proposed Basis for Tolerances of Insecticide Residues in Food Products

W. E. BAIER AND C. W. WILSON
Research Department, California Fruit Growers Exchange, Ontario, California

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The present system of insecticide residue tolerances, calculated on the "as is" weight of food, is based on the presumption that uniform protection is afforded the public and equitable consideration is given producers of various food products.

Both goals would be more nearly achieved by the proposed method. It considers non-aqueous matter as the most practical index of food consumption. It provides for a safe upper limit or tolerance based on the non-aqueous matter while retaining a nominal tolerance quite similar to present practice. It will be readily seen that the proposed basis is equally adaptable to the whole commodity as purchased or to the edible portion, whichever may be determined by future consideration.

During more than 25 years, on varoius occasions, technical workers of the California Fruit Growers Exchange have struggled with the problem of safeguarding against insecticide residue hazards, alleged or real. The experience has encompassed preparation for market and interstate shipment of both fresh citrus fruit and citrus products of various types. The following proposal is the outgrowth of that experience. Its purpose is identical with that of the Food and Drug Administration in its constant endeavor to safeguard the health of the consuming public in matters related to the use of insecticides in food production.

At first thought it would appear to the advantage of both producer and consumer if no tolerances for insecticide residues were permitted and that, if necessary for compliance with such a provision or lack of it, the use of insecticides would be abandoned. Two factors decisively mitigate against the disuse of insecticides. First, no method is yet known economically to grow crops year after year without insecticides. Without them the production goes down together with the quality of the crop with the result that the consumer must pay higher prices for mediocre value. This quality deterioration may be the direct effect of contamination of insects or insect fragments in the fresh produce or processed material, or it may result from the impaired health of the tree or plant. Secondly, safe use of insecticides is possible. The insecticide is usually applied to obtain the optimum contact with the insect pest with the least penetration or dissemination otherwise. Further, insecticides, although certainly poisonous to the pests which they are designed to kill, exhibit some lower limit related to the body weight of living organisms below which no toxic effect whatever can be demonstrated. That is, a given insecticide or poison in a certain weight per unit weight of the living organism will be toxic whereas that same insecticide at some greatly reduced level of weight per unit weight of the same living organism becomes completely non-toxic. As a matter of fact a great many poisons in extremely small amounts are actually beneficial or even essential to higher living organisms.

This tonic effect to the living organism is, of course, not the reason for insecticide tolerances. The purpose of tolerances is simply to provide a practical safeguard so that consumers, in pursuing their normal eating habits day by day, will not be subjected to amounts of insecticide residues even approaching a toxic level. Further, such tolerances should not only offer protection in normal use, but also against occasional misuse or overindulgence of particular foods at least so far as the effects of insecticide residues may be concerned.

APPROACHING THE DETERMINATION OF A TOLERANCE

If the exact eating habits of each individual could be known, hypothetically correct tolerances might be prescribed for that individual's food supplies as they differ from those of other individuals. But this is absurd and it is perfectly obvious that the nation's food supply as a whole for all the nation's consumers must be considered so far as possible in determining tolerances. The average condition must be the one which prevails in such determinations but, of course, with ample factor of safety to protect the old and weak as well as the young and strong.

As necessary as water is to the maintenance of life, no animals, not even aquatic ones, can decrease their necessary food intake to an extreme merely by filling up with water. Conversely, if all foods which are consumed were completely dehydrated, a person could not eat as much weight of these dehydrated foods as he does of the same foods in the fresh state. The reason is that the food value largely is contained in the dry matter and in the long run just so much dry matter and no more or no less is required in the diet *(1)*. It is granted that the food value expressed in calories will vary a little between carbohydrate and protein and still more between either of these and fats or fatty oils. The point is that the addition or subtraction of water does not alter the situation providing the same amount and kind of dry matter is consumed.

From a consideration of the foregoing and other factors, the basing of tolerances on the fresh weight or "as is" weight of food is not as logical, if logic only were to be considered, as would be any one of several other bases. For example, a different tolerance for each different kind and form of food or food product might be established; the tolerance might be based on the total solids; it might be based on the calorie value; on the non-volatile matter; or on the non-aqueous matter. We will not attempt to discuss the relative merits of each of these. Careful consideration of all of them, however, points to the last one as being the most meritorious, namely a tolerance of so many parts by weight of the insecticide residue per unit part by weight of the non-aqueous matter. A simple combination of this principle with the present method of expressing tolerance will be described in the following. First, however, the difficulties inherent with the present system of expressing tolerance will be described.

DIFFICULTIES WITH PRESENT METHOD OF EXPRESSING TOLERANCE

The examples taken to illustrate the shortcomings of the present method are hypothetical, but similar actual cases exist. Assume that a tolerance of a particular insecticide has been established for the fresh produce at seven parts per million (p.p.m.). It might be 5 or 10 or 100 for our example but seven is taken as a likely sounding figure. By implication or definite interpretation the same 7 p.p.m. is considered to apply to various products or by-products derived from this or other commodities in addition to applying to the fresh produce itself. In all cases the tolerance is based on "as is" weight of the particular product whether it be fresh, liquid, dry or other form. The 7 p.p.m. will have been determined as the best possible tolerance so expressed in view of the toxicological studies made, the practicability of the removal of the insecticide from the fresh produce, and its tenacity in various processing procedures. The 7 p.p.m. then represents the best obtainable single tolerance consistent with safety and practical considerations for all types of food products without necessarily being specifically best adapted to any one of these products.

If the product on which this 7 p.p.m. tolerance has been established is a fruit suitable for the production of pectin, this product might be manufactured and sold provided its content of the insecticide did not exceed seven parts per million. Some insecticides and other toxic substances are very tenaciously held by pectin, this being one reason for its value as a detoxicating agent as, for example, in treatment of diarrhea *(2, 3)*. The dry powdered pectin, as produced, might then exceed the tolerance. In such case, under the present system, it is only necessary to dissolve it in water to form the well known liquid pectin, which product would easily pass the 7 p.p.m. tolerance even though the dry pectin had been as high as 50 to 100 p.p.m. Yet, in the use of the pectin, exactly the same amount of the dry material must be used in either case so that the same amount of the insecticide residue would be contributed to the final article of food. The amount is small in any case [a] but, if there is a reasonable way to avoid it, why should there be this discrepancy or discrimination?

Certain insecticide residues are quite soluble in the essential oils, for example in oil of orange. If the 7 p.p.m. tolerance were greatly exceeded in the oil, all that would be necessary to make a marketable product well under the tolerance would be to cut the oil to derive a household extract or a flavoring emulsion. Only a few drops [b] of the oil will suffice to flavor a food composition where a teaspoon of the extract might be required; this ease of measurement and better dispersion of the extract over the oil are the advantages of the extract form of flavor to the housewife but perhaps not so desirable to the larger user. The final result as to insecticide residue entering the finished food composition is the same so why should one of these procedures be held legal and the other not?

The cutting of vinegar to make a product of lower acid strength might be considered as another example. The dilution of a fruit juice to make an ade, or the dilution of concentrated juices to make either natural strength juice or ades may be still others. All these dilution operations have their very definite place in the distribution to consumers of very desirable and convenient forms of foods and beverages but it seems very unwise that such a dilution operation should permit the production of a food satisfactory for interstate commerce from one which was not. It would seem that either both forms are satisfactory or both should be condemned depending only upon whether the insecticide residue contributed to the finished food is satisfactorily low.

Dehydrated foods may be first diluted or reconstituted with water before consumption, or they may be eaten directly, but the dehydrated food is a concentrated food and therefore the actual "as is" weight consumed in the long run will be proportionately less than the fresh foods. The same is true of non-aqueous materials such as fats and oils which are highly concentrated, high in calorie value, and therefore the amounts which can be consumed are less than those of fresh fruits and vegetables. As we have seen, the essential oils are used in extremely small quantities for another reason, to impart the required flavor. Pectin is used in small quantity to impart gel structure. The amounts of all these food products which will enter the diet are in a sense predetermined. The amounts of non-aqueous matter consumed will be essentially constant; at least these amounts will not be altered materially by adding or subtracting moisture. So it would seem that the addition or subtraction of moisture should not be a factor which determined whether a food will or will not pass an insecticide residue tolerance.

Should producers of highly concentrated or dehydrated foods, beverage bases, condiments, flavors, and food adjuncts be obliged to continue to meet a tolerance much more stringent than that for fresh fruits and vegetables, there would in time develop a consumer recognition of this. The idea would prevail that such

[a] Medicinally the maximum dosage of pectin by mouth is about 10 grams.

[b] Even in medicinal use the maximum dosage of oil is 0.3 ml.

concentrated foods would be safer to use than fresh produce. This is unavoidable under the present method yet it is highly undesirable. All classes of food must be safe and as far as possible there should not be implied different degrees of safety. The proposed scheme attempts to accomplish the desired goal.

THE PROPOSED BASIS FOR EXPRESSING TOLERANCES

Let us now assume that a new insecticide, having shown promise from the standpoint of economic entomology, is ready for consideration of tolerance. Supported by chemical and pharmacological data, the toxicity is to be determined at various residual levels in or on the fresh fruit or vegetable to which it had been applied under practical conditions of pest control. So far as possible the fruit or vegetable selected for the major study should be a variety which is capable of growing in widely separated areas and one which is readily acceptable as a substantial part of the diet of small experimental animals. The safe upper limit or tolerance, let us assume, is found to be 7 p.p.m., and let us further assume that the average non-aqueous matter in the produce in question is 14 percent. The tolerance then would be expressed on the basis of non-aqueous matter, that is, it would be expressed as 50 p.p.m. of non-aqueous matter. This is obtained by dividing 7 by 14 and multiplying by 100 to give 50 p.p.m. non-aqueous matter.

It is proposed that some figure for the fresh produce less than this 7 p.p.m., say 5 parts per million, be set as a nominal tolerance. Any food which passes this tolerance would be acceptable without further consideration no matter what the non-aqueous matter content. Should this nominal tolerance of 5 p.p.m. on the fresh weight basis be exceeded then the non-aqueous matter would be determined and if the 50 p.p.m. non-aqueous matter tolerance is not exceeded the produce or product could enter into interstate commerce.

The provision for some nominal tolerance for high moisture containing fresh produce (in this case 5 p.p.m.) is suggested as a method of expediting enforcement; fresh produce obviously well below the safe level could be passed with the minimum analytical procedure. In any event the additional analyses are not difficult in view of the now generally available laboratory comminuting devices and methods of direct titration of water content.

Table 1 gives the relationships between the present and the proposed methods of expressing tolerances assuming maximum allowable residues, in each product, of the same hypothetical insecticide used in the above examples. The various products listed could conceivably be made from citrus fruit. Any one particular insecticide would not distribute itself as to reach the tolerance in all these products, however. In this respect the table is hypothetical, serving to illustrate the proposed basis if the maximum permissible residues under its provisions were encountered.

The principle of this proposal is simple both as to concept and enforcement details. This simplicity would aid everyone concerned to know exactly what to expect and so remove any tendency of a processor, were he so minded, to laxity in insecticidal residue removal. It would go a long way toward ending the pleas for special consideration of special foodstuffs. It promises to have the added legal advantage of putting broad classifications of foods under regulations that are worded the same and mean fundamentally the same thing whether considered from the point of view of the producer, processor, consumer or law enforcement agency.

LITERATURE CITED

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TABLE 1

Hypothetical Case of a Tolerance for an Insecticide Residue of 50 p.p.m. Based on "Non-Aqueous Matter"

Citrus Food	Non-Aqueous Matter, %	Calories, per g.	Maximum Amount of Insecticide Residue			
			"As is" basis, p.p.m.[c]	Mg. per calorie	Mg. per serving (estimated)	
Single strength citrus juice	12.4	0.50	6.2	0.012	(10 oz. = 300 g.)	1.80
Oil-free 65° Brix concentrate (regular process)	64.5	2.58	32.3	0.012		1.80
Essential oil	100.0	n.d.[d]	50.0	n.d.[d]	(U. S. Disp. dose 0.3 ml. maximum)	0.01
Dried meal	88.2	3.53	44.1	0.012	Not offered as human food [e]	
Seed oil	100.0	9.00	50.0	0.006	(20 g. on salad)	1.00
"As is" dry pectin	92.5	3.70	46.3	0.012	(2 oz. gel or jelly containing 1%)	0.03
5.5 grade liquid pectin	3.0	0.12	1.5	0.012		0.03
Fresh grated peel	25.0 [g]	0.92	12.5	0.014	(1/6 oz. per pie, 1 cut)	0.01
Vinegar	6.0	n.d.[d]	3.0 [f]	n.d.[d]	(4 g. on salad)	0.01
Wine	8.0	0.32	4.0 [f]	0.012	(8 oz. = 240 g.)	1.00

[c] The figures in this column are equivalent to a non-aqueous matter tolerance of 50 p.p.m. If, in the example considered, the tolerance were expressed on the present basis each entry in this column would have been 7.0 p.p.m.

[d] n.d. = Not determined.

[e] The extent to which insecticide residues in feeds carry into those animal products used for food will, of course, depend upon the properties of a specific insecticide, hence cannot be covered in a hypothetical consideration such as this table. However, the same principle applies to feeds as to foods; it is the non-aqueous matter which is the determining factor and which should therefore become the basis of the tolerance.

[f] Equivalent to 50 p.p.m. non-aqueous matter. By combination with present tolerance basing as described in the text this figure would be 5.0.

[g] Including 2 percent oil.